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THE PLANT DISEASE REPORTER

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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion, rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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CHEMICAL CONTROL OF STEM AND LEAF RUST OF WHEAT IN KANSAS[1]

W. C. Haskett and C. O. Johnston[2]

Summary

One or two spray applications of certain fungicides reduced both leaf and stem rust (Puccinia rubigo-vera tritici, P. graminis tritici) infection in experiments conducted in Kansas during the period 1952 to 1955. In some instances losses in yield and reductions in test weight were prevented.

Chemical treatments showing some promise were Acti-dione, maneb, zineb, thiram, Karathane, and calcium sulfamate. Zineb, maneb, Acti-dione, and Karathane treatments did not adversely affect the milling and baking quality or seed viability of sprayed wheat, whereas applications of calcium sulfamate resulted in marked reduction of quality and viability.

The use of available protective fungicides on a large commercial scale is not recommended in Kansas. However, small fields of valuable seed wheat could be adequately protected.

INTRODUCTION

The two major rusts found on wheat in Kansas are leaf rust, Puccinia rubigo-vera (DC.) Wint. f. sp. tritici (Eriks.) Carleton (P. recondita Rob. et Desm.), and stem rust, P. graminis Pers. f. sp. tritici Eriks. & E. Henn. (32). These rusts may be found every year but generally do not cause an appreciable loss in the Kansas wheat crop. As in other areas, the amount of inoculum present, the weather conditions, and the receptiveness of the wheat crop all influence the development of a rust epiphytotic. No varieties of winter wheat grown in Kansas are resistant to all of the prevalent races of rust, although certain ones possess resistance to some of the major races. In view of the importance of the Kansas wheat crop and the potential damage from large-scale rust epiphytotics, chemical control experiments were initiated at Kansas State College. Many of the trials have been of a preliminary nature, and numerous experimental chemicals were tested in addition to those reported in this paper.

LITERATURE REVIEW

The earliest attempts to control cereal rusts with chemicals were reported by Kellerman (22), Pammel (33), Hitchcock and Carleton (17, 18), and Galloway (9) from 1891 to 1894. Although these early investigators obtained some control, the equipment and chemicals available made the method impractical.

A revival of interest in this problem was evident from 1925 to 1934 in the wide experimental use of sulfur dusts to control the cereal rusts. Promising results by dusting sulfur on small grains were demonstrated by Kightlinger (23), Kightlinger and Whetzel (24), Bailey and Greaney (2, 3, 4), Petri (34), Greaney (11, 12, 13), Lambert and Stakman (25, 26), and Craigie and Greaney (7). The results of these workers indicated that, by applying sulfur to wheat, fairly good control could be obtained. These sulfur applications were made by both ground and airplane dusting equipment. Despite moderate success, sulfur was not considered economical for large-scale rust control operations in the wheat growing regions.

Later Hart and Allison (14) were able to control stem rust by soil applications of sodium borate (Borax -- sodium tetra-borate), picric acid, and para and ortho toluolsulfonamides. Their methods, although effective, required too heavy a rate of application to be economical.

In Germany, Gassner and Hassebrauk (10) tested approximately 300 chemicals for their

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[2]Formerly Associate Pathologist and Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, respectively.

effect on various cereal rusts. Chemicals were added to the soil at the time of rust infection. Effectiveness of the chemicals was measured by the changes in rust reaction. They found that acridin, 1-bromo-2-naphthol, alpha-naphthol, nitrodibromobenzol, picric acid, para-toluol-sulfochloramide--sodium salt, and 3-4-6 tribrom-1-2-naphthoquinone were the most effective chemicals tested.

Later Hassebrauk (15) found that of the chemicals tested by Gassner and Hassebrauk (10) the most effective were picric acid, trinitrotoluene, p-toluolsulfochloramide--sodium salt, and p-toluolsulfonamide.

In 1951 Hassebrauk (16), working with four different cereal rusts, observed that para-amino benzol sulfonamide, acetyl sulfanilamide, 2-para-amino benzol sulfonamido-pyridine, 6-sulfanilamino-2,4-dimethyl pyridine, and saccharin gave some measure of control. These chemicals were applied to the soil at the time of rust infection.

Alteration of stem rust reaction was obtained by Johnson (21), who reported the change from resistance to susceptibility in Khapli wheat caused by the use of DDT.

The effectiveness of soil applications of sulfonamide derivatives was confirmed by Mitchell, Hotson, and Bell (31). They were able to reduce rust infection with sulfanilamide and several of its derivatives. Sulfadiazine was most effective; sulfapyridine, sulfanilamide, sulfathiazole, and sulfamerizine were less so. Amate when used at a subphytotoxic level prevented rust development in wheat leaves.

Hotson (19, 20) demonstrated that certain of the sulfa drugs, including sulfamerizine, sulfadiazine, sulfapyrazine, sulfapyridine, sulfaphridazine, gantrisin, and 3-sulfanilamide 6-methyl pyridazine, at rates as low as 5 pounds per acre would control wheat stem rust. Hotson also found that this control may be counteracted by para-amino benzoic acid or folic acid.

Dickson, Grimm, and Hooker (8) reported that certain naphthoquinones and phenols have fungicidal value in low concentrations in the control of cereal rusts. They believed that ethyl alcohol solution of technical 2,3-dichloro-1,4-naphthoquinone (Phygon) with a stabilizer was the most economical compound available.

Livingston (28) tested 179 chemicals to determine their value in the control of leaf rust of wheat. Calcium sulfamate was the most effective in the group tested. The only phytotoxic effect observed was a decrease in seed germination. Calcium sulfamate demonstrated only a limited ability to protect wheat plants. Acti-dione was observed to be a good eradicant and also to protect the plants. However, the range between fungicidal effectiveness and phytotoxicity was very narrow. Mattern and Livingston (29) reported no adverse effects on baking behavior of wheat treated with Acti-dione or sodium sulfanilate. Calcium sulfamate induced properties in the harvested seeds which were detrimental to baking properties.

Levine (27), Brentzel (5), and Semeniuk (35) obtained good control with calcium sulfamate but observed some injury. Brentzel (6), working in North Dakota, reported increased yields and test weights with calcium sulfamate and sodium sulfanilate and combinations of the two chemicals. Generally these treatments gave slight decreases in seed germination. He recommended that calcium sulfamate be applied before heading and after grain maturity. The greatest effect on germination occurred when the calcium sulfamate was applied at flowering time.

Acosta (1) obtained increased yields by spraying wheat and oats with calcium sulfamate. Stem rust was substantially reduced on each crop. Sodium sulfanilate increased yields, reduced stem rust, and caused no apparent injury to the wheat. Calcium sulfamate applied at the tillering stage reduced yield. Applications following pollination reduced seed germination.

Brentzel (6) further reported that liquid Parzate and Dithane with zinc sulfate added might be used during severe rust outbreaks. Karathane gave some measure of control with airplane applications but the increase in yield did not justify the use of this compound. He concluded that newer fungicides show marked improvement over sulfur dusts but still leave much to be desired.

EXPERIMENTAL METHODS

The experiments reported in this paper were conducted at the Kansas State College agronomy farm, Manhattan, Kansas.

Pawnee winter wheat was used in the 1952 test; Cheyenne winter wheat in the 1953, 1954, and 1955 tests. The spacing between rows was 12 inches, except in 1952 when a 10-inch drill row was used. Each plot was 4 drill rows wide and 20 feet long. The two middle rows,

trimmed to 16 feet in length, were harvested for yield and other information. Four replications were used in 1952 and 1954; three replications in 1953 and 1955.

The chemicals were applied with Hudson knapsack sprayers and a Niagara Cyclone hand duster. No attempt was made to apply the chemicals at a certain stage of wheat development. Generally, rust had appeared in the plots before the first application was made. This was not possible in 1953 when stem rust made a very late appearance in Kansas.

The various fungicides used and the active ingredients in each are shown in Table 1 according to McCallan, et al. (30).

The leaf and stem rust in 1952 and 1953 resulted from natural infection. The rust in 1954 and 1955 spread from "rust spreader rows" which were inoculated by means of hypodermic injections of urediospores in water.

Table 1. Chemical names for active ingredients of fungicides used in cereal rust control at Manhattan, Kansas, 1952-55.

Fungicide[a]	Active ingredient
Acti-dione (cycloheximide)	3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide
BB-360 (merbam 2)	Phenyl mercury dimethyl dithiocarbamate
Calcium sulfamate	Calcium sulfamate
Dithane D-14 (nabam)	disodium ethylenebis [dithiocarbamate]
Dithane Z-78 (zineb)	zinc ethylenebis [dithiocarbamate]
HD-160	sodium 2-benzothizylthioglycollate
Karathane	2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and isomers
Manzate (maneb)	manganous ethylenebis [dithiocarbamate]
Orthocide 406 (captan)	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide
Phygon (dichlone)	2,3-dichloro-1,4-naphthoquinone
Sulfuron	sulfur
Thylate (thiram)	bis (dimethylthiocarbamoyl) disulfide
Vancide 51	sodium dimethyl dithiocarbamate; sodium derivative of 2-mercaptobenzothiazole

[a]The authors wish to thank the following manufacturers for supplying free chemical samples: California Spray Chemical Corp., E. I. du Pont de Nemours Co., Rohm and Haas Co., The Upjohn Co., R. T. Vanderbilt Co., and Chipman Chemical Co.

EXPERIMENTAL RESULTS

1952 Test

The treatments in 1952 consisted of five sprays, five dusts, and an untreated control. The dates of application were May 20, May 26, and June 6.

Fairly heavy leaf rust developed with approximately 75 percent severity on the untreated wheat, but only a trace of stem rust developed in the plots.

All treatments reduced the leaf rust infection to some degree. The calcium sulfamate spray was the most effective, followed by maneb dust, sulfur dust, zineb dust, and zineb spray. The amount of rust infection, yields, and test weights resulting from the various treatments are presented in Table 2.

Table 2. Effect of fungicides on yield, test weight, and rust infection of Pawnee wheat at Manhattan, Kansas, 1952.

Treatment	Rate per acre[a]	Leaf rust %	Yield Bu./A.	Test weight Lb./Bu.
Control	None	65.0	60.2	61.4
Sulfur dust	40	10.0	61.2	61.8
Zineb dust	40	22.5	59.5	62.0
Maneb dust	40	6.2	53.7	62.0
Phygon dust	40	52.5	47.7[b]	61.4
Calcium sulfamate dust	40	37.5	49.2[b]	60.9
Zineb spray	1 1/2-100	20.0	63.1	61.6
Maneb spray	1 1/2-100	30.0	57.9	61.9
Phygon spray	1/4-100	57.5	62.8	62.0
Calcium sulfamate spray	5-100	.2	43.5[c]	52.8[c]
Orthocide 406 spray	2-100	50.0	63.8	62.0
L.S.D. .05		--	10.1	1.1
L.S.D. .01		--	13.5	1.4

[a]Rate of spray applications expressed as pounds of active material in gallons of water per acre. Rate of dust applications expressed as pounds of formulated material per acre.
[b]Significant differences between treated and untreated plots at .05 level.
[c]Significant difference between treated and untreated plots at .01 level.

There were no significant increases in yield or test weight over the untreated control. Treatments with Phygon and calcium sulfamate dusts resulted in significant yield reductions. The calcium sulfamate spray caused a highly significant reduction in both yield and test weight. The wheat sprayed with calcium sulfamate showed definite injury expressed as leaf burning after the second application. The grain was shriveled and had a burnt caramel odor.

The 1952 experiment revealed that certain fungicides were effective in reducing the severity of leaf rust infection. However, the early maturity of the wheat and the absence of stem rust did not permit a complete evaluation of the treatments.

1953 Test

The experiment in 1953 included four dust and seven spray treatments and the untreated control. Applications were made May 15, May 25, and June 5. The first stem rust was observed June 4 and was well established by June 15. Hot, dry winds in mid-June caused the Cheyenne wheat to mature early and stopped all rust development. An early stem rust evaluation was made by counting the number of stem rust pustules on 100 flag leaves in each plot. The stem rust reading for each treatment recorded in Table 3 is the average number of stem rust pustules per flag leaf. As shown in Table 3, the treatments giving the greatest reduction in stem rust were maneb dust and spray, zineb dust and spray, and the calcium sulfamate sprays. As in 1952, there were no significant increases in yield or test weight. Calcium sulfamate caused significant reductions in both yield and test weight. The wheat did not appear to be so severely injured as in 1952. The two calcium sulfamate sprays reduced germination by 45 and 32 percent. As in 1952, Phygon spray caused a significant yield reduction.

Early maturity of the wheat and inhibition of stem rust development by hot dry weather made it difficult to judge the effectiveness of the various chemicals in controlling stem rust.

Table 3. Effect of fungicides on the yield, test weight, seed germination, and rust infection of Cheyenne wheat at Manhattan, Kansas, 1953.

Treatment	Rate per acre[a]	Stem rust No.[b]	Yield Bu./A.	Test weight Lb./Bu.	Germination %
Control	None	5.28	28.3	56.7	84.0
Sulfur dust	40	.69	32.7	57.3	90.0
Calcium sulfamate dust	40	1.65	25.6	56.3	90.7
Maneb dust	40	.01	29.3	57.3	82.7
Zineb dust	40	.29	27.9	56.8	83.3
Zineb spray	1 1/2-100	.12	28.9	58.0	88.6
Maneb spray	1 1/2-100	.03	28.8	57.2	91.3
Vancide 51 spray	2-100	4.28	28.2	57.3	84.0
Calcium sulfamate spray	5-100	.04	22.1[c]	52.5[c]	46.7
Calcium sulfamate spray	12.5-10	.18	23.2[d]	52.5[c]	57.3
Orthocide 406 spray	2-100	1.43	26.1	57.0	88.0
Phygon XL spray	1/4-100	1.63	23.4[d]	56.2	89.3
L.S.D. .05		--	4.6	1.8	--
L.S.D. .01		--	6.1	2.4	--

[a]See Table 2 footnote[a].
[b]Stem rust value expressed as the average number of stem-rust pustules on each flag leaf.
[c]Significant difference between treated and untreated plots at .01 level.
[d]Significant difference between treated and untreated plots at .05 level.

1954 Test

In the 1954 test, only six sprays, one dust, and the untreated control were used. The spray treatments were made at 10 gallons of water per acre in an attempt to simulate low gallonage airplane spraying. One and two applications of calcium sulfamate, maneb, zineb, and Karathane were made. Two applications of BB360, HD160, and sulfur dust were made. The first application was May 16 and the second June 2. Rust development was heavy enough to give a reading of 62.5 percent for leaf rust and 47.5 percent for stem rust on the sprayed control plots, as shown in Table 4. The most effective treatments were two applications each of Karathane, maneb, and zineb. These treatments gave increases of 4.2, 3.7, and 2.2 bushels per acre, respectively, over the untreated wheat. In all cases two applications gave better rust control than one application. Except in the case of calcium sulfamate, plots receiving two applications also yielded more than those receiving one. Plots receiving two applications of calcium sulfamate showed the greatest reduction in leaf and stem rust but produced 3.2 bushels per acre less grain than untreated wheat. Two application of zineb and Karathane gave significant increases in test weight. Calcium sulfamate sprays caused a slight reduction in test weight but very little reduction in yield. However, the germination of grain from calcium sulfamate-treated plots was definitely lower than that from other plots. There was no noticeable difference in the appearance or odor of the grain.

The 1954 test indicated that zineb, maneb, and Karathane sprays were effective in reducing rust infection, resulting in slightly higher yields of grain having higher test weight than unsprayed wheat.

Table 4. Effect of fungicides on the yield, test weight, seed germination, and rust infection of Cheyenne wheat at Manhattan, Kansas, 1954.

Treatment	No. of applications	Rate per acre[a]	Leaf rust %	Stem rust %	Yield Bu./A.	Test weight Lb./Bu.	Germination %
Control	-	None	62.5	47.5	33.0	55.1	93.2
Sulfur dust	2	40	55.0	43.8	32.2	54.6	94.1
Calcium sulfamate spray	1	12.5-5	52.5	42.5	31.7	54.0	87.5
Calcium sulfamate spray	2	12.5-5	4.0	11.8	29.8	54.4	79.6
Maneb spray	1	5-10	27.5	32.5	33.8	55.4	95.3
Maneb spray	2	5-10	11.2	17.5	36.7	57.8[b]	94.7
Karathane spray	1	5-10	47.5	36.2	30.4	54.0	91.0
Karathane spray	2	5-10	41.2	21.2	37.2	57.4[c]	92.0
Zineb spray	1	5-10	57.5	43.8	33.3	55.5	89.8
Zineb spray	2	5-10	27.5	13.8	35.2	56.9	94.4
HD 160 spray	2	5-10	45.0	32.5	30.2	56.3	91.6
BB 360 spray	2	5-10	55.0	40.0	32.5	54.9	92.8
L.S.D. .05			--	--	4.4	2.0	--
L.S.D. .01			--	--	5.8	2.6	--

[a]See Table 2
[b]Significant difference between treated and untreated plots at .01 level.
[c]Significant difference between treated and untreated plots at .05 level.

1955 Test

The 1955 test consisted of seven chemical sprays and the untreated control. One application was made June 5 when the Cheyenne wheat was in the early soft dough stage. Only a trace of leaf rust developed in this nursery. Stem rust developed well and reached a severity of 41.6 percent on the untreated wheat. Infection on the upper internodes predominated.

All treatments gave excellent control of stem rust. The tank mix of nabam and zinc sulfate at both high and low dosage rates was very effective. Acti-dione, calcium sulfamate, maneb, thiram, and Karathane all produced marked reductions in stem rust. All treated plots had higher yields than the untreated wheat. The yield differences and stem rust control are indicated in Table 5.

For the first time during the course of these experiments, plots treated with calcium sulfamate gave higher average yields than the untreated control. This may have been due to the low concentration of the chemical in the spray which, while giving good rust control, did not result in plant damage. There was little variation in test weight and only a slight decrease in seed germination due to calcium sulfamate spray.

No phytotoxic effects were observed in the Acti-dione- or calcium sulfamate-treated wheat, nor was there any noticeable difference in the appearance or odor of grain from treated and untreated plots.

Table 5. Effect of fungicides on yield, test weight, seed germination, and rust infection of Cheyenne wheat at Manhattan, Kansas, 1955.

Treatment	Rate per acre	Stem rust %	Yield Bu./A.	Weight Lb./Bu.	Seed germination %
Control	None	41.6	33.2	60.5	92.7
Nabam + zinc sulfate	2/3-4-40[a]	3.0	36.1	60.8	93.5
Nabam + zinc sulfate	4-1 1/2-40	2.0	35.9	61.0	91.2
Acti-dione	100 ppm. -40	1.0	36.8	60.2	89.5
Calcium sulfamate	1-40	2.3	38.1[b]	60.2	86.1
Maneb	1-40	8.3	36.1	60.5	93.2
Thylate	1-40	11.7	39.9[c]	60.5	91.2
Karathane	1-40	4.3	34.1	60.7	90.5
L.S.D. .05[b]		--	4.8	.8	--
L.S.D. .01[c]		--	6.7	1.1	--

[a]Pounds of nabam to pounds of zinc sulfate to gallons of water per acre.
[b]Significant difference between treated and untreated plots at .05 level.
[c]Significant difference between treated and untreated plots at .01 level.

Table 6. Effect of cereal rust fungicides on the milling, chemical and baking quality of Cheyenne winter wheat grown at Manhattan, Kansas, in 1954 and 1955.[a]

Treatment	Wheat				Flour		Bread baking data[b]						
	Wt. per bu. lbs.	Ash %	Protein %	Flour yield %	Ash %	Protein %	Absorption %	Mixing time min.	$KBrO_3$ Requirement mg.	Crum Grain	Crum Color	Loaf volume (cc) As Rec'd	Loaf volume (cc) Corrected
1954													To 11.5%
Control	55.6	1.79	11.6	73.3	.45	10.9	60.5	5	1	S	S	795	836
Calcium sulfamate	53.3	1.95	13.0	71.3	.58	12.5	60.5	2 7/8	3	Q-U	S	813	758 U
Maneb	57.2	1.83	12.6	74.4	.45	11.8	61.0	4 3/4	1	S	S	843	823
Karathane	57.0	1.76	12.6	74.9	.43	11.8	62.2	4 7/8	1	S	S	837	818
Zineb	56.2	1.77	12.4	73.0	.41	11.1	61.0	5	1	S	S	810	837
1955													To 10.5%
Control	60.6	1.44	11.9	74.4	.42	11.0	61.1	4 1/4	1	S	S	795	762
Acti-dione	60.4	1.49	11.2	75.3	.33	10.4	60.2	4	1	S	S	777	784
Calcium sulfamate	60.4	1.45	11.5	76.7	.46	10.8	60.5	3	3-4	Q	U	798	777

[a]Chemical data expressed on a 14% moisture basis.
[b]S, Q, and U = Satisfactory, questionable, and unsatisfactory.

MILLING AND BAKING TESTS

To determine the effect of some of the more promising chemicals on milling and baking quality, samples of grain were submitted to K. F. Finney, Chemist, Hard Winter Wheat Quality Laboratory. His complete report giving chemical, milling, and baking data is presented in Table 6.

Wheat harvested from plots treated with maneb, zineb, and Karathane in 1954, and Actidione in 1955, appeared normal in every respect. It is pertinent to note that calcium sulfamate shortened mixing time and decreased mixing tolerance and stability. It was necessary to use about three times the usual quantity of potassium bromate to obtain loaf volumes equal to those for the other treatments in 1955. Crumb characters in bread made from grain from calcium sulfamate treated plots were questionable or unsatisfactory. The corrected loaf volume was rated as unsatisfactory in 1954. The impaired condition of the crumb grain indicated that a permanent type of injury resulted from the use of calcium sulfamate.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH KANSAS AGRICULTURAL EXPERIMENT STATION

NEW PHYSIOLOGIC RACES OF TILLETIA CARIES IN THE PACIFIC NORTHWEST[1]

E. L. Kendrick and C. S. Holton[2]

In the Pacific Northwest pathogenic specialization in Tilletia caries (DC.) Tul. and T. foetida (Wallr.) Liro is the major factor contributing to the problem of controlling wheat smut by growing resistant varieties. Thus, the need for information on the number, kinds, and distribution of races is self-evident. To keep abreast of this problem, therefore, an annual survey is made by testing approximately 200 collections of smut from commercial wheat fields throughout the region on a standard set of differential host varieties. The methods and tester varieties that are used are the same as those used by other workers (3, 5), except that the variety Orin was substituted for Oro and two new varieties were added. The additional varieties were Sarheim (Hussar x Hohenheimer, C. I. 10068-1) and Omar (C. I. 13072). The latter variety was released for commercial production in 1955 and was resistant to all races of smut known at that time.

Upwards of 500 collections of smut, representing 3 crop years, were tested during the period 1954 through 1957, and only two new races were identified. These are designated as T-17 and T-18. Their comparative reactions, together with the reactions of seven previously known races (6), are shown in Table 1. Race T-17 was from a commercial field of Elgin wheat near Helix, Oregon, and T-18 came from a farmer's seed increase field of Omar near Latah, Washington. Only one of the new races, T-18, represents a distinctly new pathogenic type.

As shown in Table 1, it has been possible in the past to obtain resistance to all known races with any combination of the Martin, Turkey, and Ridit types of resistance, since those races attacking one type of resistance are unable to attack either of the other two (4). Both the Martin and Turkey types of resistance represent combinations of dominant factors for resistance (1), while Ridit resistance represents a combination of recessive factors (2). Therefore, from a breeding standpoint, a combination of the Martin and Turkey types of resistance introduces fewer problems than any other combination in breeding towards resistance to all races.

The Turkey-Martin combination of resistance factors is primarily responsible for the high resistance of Omar, a soft white club wheat that is the first commercial variety to combine these resistance factors successfully. It remained smut-free throughout 10 years of breeding and selection trials involving tests for resistance to all known races of bunt, and is the most highly resistant variety ever to be released in the Pacific Northwest. Thus, the discovery of race T-18 is a matter of major importance, since this race is able to attack varieties possessing both the Turkey and Martin types of resistance (Table 1). In this connection it is significant that Albit and White Odessa, each of which possesses only the M_1 gene of the Martin complex (M_1M_2), are highly susceptible to T-18, while Martin and Omar, which possess both the M_1 and M_2 genes, display partial resistance to this new race, as indicated by an intermediate reaction. Apparently the M_2 gene provides Omar's weak resistance to T-18. Experience has shown, however, that weak resistance to smut is inadequate under the conditions of wheat production in the Pacific Northwest. Consequently, it will be necessary to utilize other kinds of resistance in breeding programs to develop new varieties that will provide protection from T-18. It is clear from the reactions shown in Table 1 that the Ridit resistance is effective against this new race, and this may be the most convenient immediately available source of resistance to it.

The reaction chart (Table 1) shows that the other new race (T-17) and one of the old races (L-8) are identical in their host range, and, therefore, T-17 presents no new problem in the breeding of smut-resistant varieties. These races are distinct, however, in two respects. In the first place, they represent different species, T. caries and T. foetida, respectively. Moreover, they differ in the important respect of effect on host vigor. Race L-8, which has long been used as the tester to identify the Turkey (T) type of smut resistance in

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[2] Pathologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Pullman, Washington.

Table 1. Reactions of differential wheat varieties to certain physiologic races of Tilletia caries and Tilletia foetida.

	Tester varieties, resistance factors[a], and differential reactions[b]									
Race[c]	Hybrid 128 -	Ridit Rd	Orin T	Albit M_1	Hohenheimer Ho	Hussar Hu M_1	Martin M_1M_2	White Odessa M_1	Sarhein Hu Ho	Omar M_1M_2 T
T-1	S	R	R	R	R	R	R	R	R	R
T-6	S	R	R	S	R	R	S	S	R	R
T-8	S	R	R	S	R	S	S	S	R	R
T-12	S	R	R	S	S	R	I	S	R	R
T-13	S	S	R	S	R	S	R	S	R	R
T-16	S	R	S	R	S	R	R	R	I	R
L-8	S	R	S	R	R	R	R	R	R	R
T-17	S	R	S	R	R	R	R	R	R	R
T-18	S	R	S	S	R	R	I	S	R	I

[a] Designated by symbols representing variety source: Rd = Ridit; T = Turkey; M_1M_2 = Martin; Ho = Hohenheimer; Hu = Hussar.
[b] R = Resistant (0 - 10 percent infection); I = Intermediate (11 - 40 percent infection); S = Susceptible (41 - 100 percent infection).
[c] "T" and "L" races denote T. caries and T. foetida, respectively.

hybrid populations, is undesirable for this purpose because it may weaken the vigor of infected plants beyond the point of survival. This frequently results in depleted stands, thereby complicating analysis of genetic populations. The new race, T-17, is significantly less debilitating to the infected plant and consequently causes little or no reduction in stand. Thus, T-17 may be preferable to L-8 as a tester race for smut resistance in genetic populations.

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UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, AND AGRICULTURAL EXPERIMENT STATIONS OF IDAHO, OREGON AND WASHINGTON

A NEW GRASS HOST FOR DWARF BUNT

W. G. Dewey and D. C. Tingey[1]

Several plants of goatgrass (Aegilops cylindrica Host.) exhibiting typical dwarf bunt symptoms were collected near Nephi, Utah, during the summer of 1955. The infected plants were growing along a roadside adjacent to a field of Utah Kanred wheat in which a considerable amount of dwarf bunt was observed.

Infected culms showed characteristic dwarfing, the spikes were more compact than normal and typical hard, dark sori were borne in the distended florets (Fig. 1).

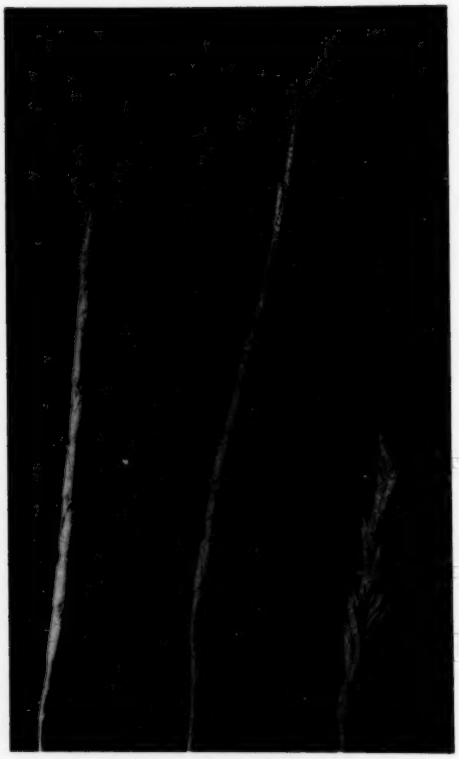

FIGURE 1. Healthy and infected spikes of Aegilops cylindrica. Left to right: a healthy spike; an occasional infected floret; a severely infected spike.

A microscopic comparison of spores of the causal organism (Tilletia contraversa Kuehn) taken from goatgrass and from wheat revealed no distinguishable morphological differences. In both instances the spores were deeply reticulate and surrounded by a hyaline sheath.

This occurrence of dwarf bunt in goatgrass apparently involves the extension of the known host range of T. contraversa into a new genus.

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With this addition, the known host range of this fungus now includes the following ten genera (1, 2, 3).

Aegilops	Elymus
Agropyron	Festuca
Arrhenatherum	Lolium
Bromus	Secale
Dactylis	Triticum

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SEPTORIA REACTIONS OF OAT VARIETIES AND SELECTIONS IN 1957[1]

A. L. Hooker[2]

Abstract

Three hundred sixty-one oat varieties or new experimental selections were artificially inoculated with Septoria avenae in replicated experiments at Madison, Wisconsin, in 1957. Significant differences in varietal reaction were measured in percentage of leaf, stem, and kernel infection. The oat strains varied widely in degree of infection. Several had low disease scores.

The present report is the third in a series (1, 3) on the Septoria reactions of presently important or recently important commercial oat varieties and of new experimental selections at Madison, Wisconsin. In 1957, the leaf, stem, and kernel reactions to Septoria avenae Frank were determined for 361 oat varieties or selections in an artificially inoculated Septoria nursery.

MATERIALS AND METHODS

Three replications of the 361 oat strains were sown on April 23 and 24 in small plots in a randomized complete block design. All plots were artificially inoculated by spraying with a water suspension of spores and mycelial segments of the pathogen on June 5, 10, 13, and 20. Kernel infections were induced by injections of inoculum around the developing panicle with a hypodermic needle, while the plants were in the boot stage.

A composite of nine cultures of S. avenae was used for inoculation in 1957. These were selected, on the basis of sporulation and pathogenicity, from among numerous cultures which had been isolated and tested for pathogenicity in the greenhouse. As a group, they may have been somewhat more virulent than the cultures used in 1955 or in 1956 (1, 3).

Leaf infection scores, recorded as an estimated percentage of leaf area infected on a whole plant basis, were taken on July 8. Ten culms, at random, were harvested from each plot at maturity. These were rated, on a visual basis, in the laboratory for percentage of stem area infected after the leaves and leaf sheaths had been removed. Kernel infection scores were obtained on the threshed seed from the hypodermically inoculated plants.

The data obtained were subjected to statistical analyses.

Additional details regarding inoculation procedures and methods of recording varietal reaction have been published (2).

RESULTS

A severe epiphytotic of the Septoria disease developed on the leaves of susceptible varieties in the inoculated nursery. Only a moderate amount of natural infection occurred, however, in adjacent non-inoculated plots. Subsequent spread of the fungus to the stems resulted in heavy but differential culm infections. Up to 40 percent of the kernels in susceptible varieties were infected following the hypodermic inoculations. The grain ripened without lodging.

Varietal differences in reaction to S. avenae in the leaf, stem, and kernel were measured among the varieties (Table 1) and among the experimental selections in 1957. These differences were all highly significant statistically. Calculated L.S.D. values for leaf, stem, and kernel infection, respectively, were 6.4, 17.7, and 14.1 at the 5% level and 8.4, 23.3, and 18.6 at the 1% level.

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[1]Cooperative investigation between the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Madison, Wisconsin. The author is indebted to R. W. Ruhde for technical assistance.

Table 1. Leaf, stem, and kernel reactions to Septoria avenae of 43 oat varieties at Madison, Wisconsin, in 1957 and 2- or 3- year average for leaf, stem, and kernel infection. Leaf and stem ratings followed spray inoculations, and kernel ratings followed hypodermic inoculations. Average of 3 replications.

Variety	C.I. No.[a]	Percentage of infection						
		1957 tests			Two-or three-year average			
		Leaf	Stem	Kernel	No. years[b]	Leaf	Stem	Kernel
Ajax	4157	33	12	1	3	30	20	2
Andrew	4170	32	48	11	3	37	53	6
Beedee	6752	35	20	40	3	34	19	31
Bentland	6930	33	32	17	3	40	42	17
Bond	2733	33	27	14	2	36	41	10
Branch	5013	32	22	20	3	31	21	15
Burnett	6537	33	42	4	3	37	34	5
Cherokee	5444	35	33	10	3	39	33	6
Cherokee Reselect	7194	42	58	10	2	46	64	9
Clarion	5647	32	57	5	3	37	60	11
Clintafe	5869	25	25	12	3	30	18	8
Clintland	6701	28	23	12	3	29	16	12
Clinton	3971	33	17	5	3	32	10	5
Clinton 59	4259	25	12	6	3	31	8	10
Columbia	2820	28	57	9	2	33	34	6
Craig	5332	30	27	9	2	32	18	6
Dupre	4672	30	32	22	2	34	22	12
Early Clinton	7192	33	37	12	2	38	34	8
Fayette	6916	52	35	25	3	45	48	33
Fundy	7288	25	10	13	-	-	-	-
Garry	6662	33	33	21	3	36	34	11
Gopher	2027	30	15	11	3	34	9	5
Jackson	5441	32	22	18	3	35	22	7
Kanota	839	25	17	7	2	28	14	10
Logan	6929	33	42	22	3	40	50	14
Minhafer	6913	32	25	6	3	35	24	7
Minland	6765	32	37	15	3	40	36	20
Mo. 0-205	4988	27	30	5	3	33	35	13
Mohawk	4327	28	18	5	2	31	12	3
Nemaha	4301	35	35	8	3	37	34	15
Newton	6642	35	45	12	3	38	51	15
Osage	3991	35	35	18	2	35	31	14
Putnam	6927	35	33	40	3	40	31	36
Rodney	6661	28	40	2	3	34	48	8
Roxton	4134	27	23	2	2	31	18	2
Sauk	5986	28	32	10	3	32	32	13
Scotian	7203	27	40	3	2	31	31	2
Shield	7209	35	27	12	-	-	-	-
Simcoe	6767	28	27	4	3	30	22	10
State Pride	1154	33	25	3	3	36	24	3
Tama	3502	37	22	18	2	36	20	19
Vicland	3611	42	12	14	3	40	11	13
Victory	560	23	23	4	2	30	14	4

[a] Refers to accession number of Cereal Crops Research Branch, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[b] Two-year average refers to 1956 and 1957 tests; three-year average to 1955, 1956, and 1957 tests.

Varietal reaction to S. avenae in 1955 was similar to that in 1956 (4). A comparison of the 1957 ratings with the 2- or 3-year average infection (Table 1) indicates that a similar ranking of the varieties was measured in 1957.

On the basis of the 2- or 3-year average, Ajax, Clintafe, Clintland, Kanota, Simcoe, and Victory had the least leaf infection; and Bentland, Cherokee Reselect, Fayette, Logan, Minland, Putnam, and Vicland had most. Stem infection scores for the period were the lowest on the Ajax, Beedee, Clintafe, Clintland, Clinton, Clinton 59, Craig, Gopher, Kanota, Mohawk, Roxton, Tama, Vicland, and Victory varieties and highest on Andrew, Bentland, Bond, Cherokee Reselect, Clarion, Fayette, Logan, Newton, and Rodney. The varieties Beedee, Fayette, Minland, and Putnam showed considerable kernel infection, while an average of 5 percent or less kernel infection was found on the Ajax, Burnett, Clinton, Gopher, Mohawk, Roxton, Scotian, State Pride, and Victory varieties. Ajax, Clintafe, Clintland, Clinton 59, Craig, Gopher, Kanota, Mohawk, Roxton, and Victory had the lowest combined leaf, stem, and kernel infection scores for the testing period, while Cherokee Reselect, Clarion, Fayette, Logan, Newton, and Putnam had the highest combined infection.

Over 300 experimental oat selections were also evaluated for reaction to Septoria. Selections from the following crosses had low or high Septoria leaf and stem infection in 1957:

Low Leaf Infection (28% or Less)

(Bonda x Hajira-Joanette-Santa Fe) x Branch

C.I. 5190 x (Clinton^{2} x Santa Fe)

Clintland^{2} x ((Clinton 59^{7} x Landhafer)^{4} x (Clinton-B-C x RL 2105))

Clintland x (Garry x Hawkeye-Victoria)

Clinton x Boone-Cartier

Clinton^{4} x Santa Fe

(D69 x Bond) x (Victoria-Richland x Bannock)

Erban x (Richland x Bond)

(Erban x (Richland x Bond)) x (Landhafer x (Forward x C.I. 5544))

Exeter x Roxton

(Forward x Victoria-Richland) x Andrew

Garry x (((Forward x Victoria-Richland) x Vicland) x (Hawkeye x Victoria))

Goldwin x (C.I. 4192 x Victoria-Rainbow)

(Landhafer x (Bond-Rainbow x Hajira-Joanette))x Andrew

(Landhafer x (Bond-Rainbow x Hajira-Joanette))x Clinton

(Landhafer x (Forward x C.I. 5544)) x (Richland x Bond)

(Landhafer x (Mindo x Hajira-Joanette)) x Andrew

(Landhafer x (Mindo x Hajira-Joanette)) x Clinton

(Markton x Iogold) x (Bonda x Hajira-Joanette-Santa Fe)

Mo. 0-205 x (Bonda x Hajira-Joanette x Santa Fe)

(Ped. 19 x Bond) x (Garry x (Hawkeye x Victoria))

(Richland x Bond) x (Garry x (Hawkeye x Victoria))

Rodney x C.I. 4795

Rodney x (Landhafer x (Forward x Victoria-Richland))

Vicland x (Landhafer x Richland-Bond)

((Victoria x Hajira-Bannock) x (Victoria-Hajira x Ajax)) x Mo. 0-205

High Leaf Infection (45% or More)

Osage x ((Bonda x Hajira-Joanette) x Santa Fe)

Rodney x (Landhafer x Forvic)

Rodney x (Landhafer x (Forward x Victoria-Richland))

Vicland^2 x C.I. 5323

Vicland^2 x (Landhafer x (Forward x Victoria-Richland))

Low Stem Infection (15% or Less)

Clintland x (Garry x Hawkeye-Victoria)

(Clinton Reselect3 x Santa Fe) x Clinton

Erban x (Richland x Bond)

Garry x (((Forward x Victoria-Richland) x Vicland) x (Hawkeye x Victoria))

(Landhafer x (Bond-Rainbow x Hajira-Joanette)) x Andrew

(Landhafer x (Bond-Rainbow x Hajira-Joanette)) x Clinton

(Landhafer x (Mindo x Hajira-Joanette)) x Andrew

Landhafer x (Richland x Bond)

Vicland x (Landhafer x Richland-Bond)

(Victory x Hajira) x (D69 x Bond)

High Stem Infection (50% or More)

Ajax x (Hawkeye x Victoria)

Bonda x (Landhafer x (Forward x Victoria-Richland))

(Clarion x Bonda) x (Hajira-Joanette x Santa Fe)

Clintland x (Garry x Hawkeye-Victoria)

(Garry x (Hawkeye x Victoria)) x (Forward x C.I. 5544) x Andrew)

(Garry x (Hawkeye x Victoria)) x Vicland

(Hajira-Joanette x Bond-Rainbow x Santa Fe) x (Andrew x Landhafer)

(Landhafer x (Bond-Rainbow x Hajira-Joanette)) x Andrew

(Landhafer x (Mindo x Hajira-Joanette)) x Andrew

Mo. 0-205 x (Bonda x Hajira-Joanette x Santa Fe)

(Osage x ((Bonda x Hajira-Joanette) x Santa Fe)

Osage^2 x (Overland x Bonda x Hajira-Joanette)

(Richland x Bond) x (Garry x (Hawkeye x Victoria))

Vicland^2 x (Landhafer x (Forward x Victoria-Richland))

Selections from the following crosses had low Septoria leaf and stem infection scores for 2 or more years:

Leaf Infection 30% or Less

Clintland x (Garry x Hawkeye-Victoria)

Clinton[4] x Santa Fe

(D69 x Bond) x (Victoria-Richland x Bannock)

Forvic x (Hawkeye x Victoria)

(Landhafer x (Mindo x Hajira-Joanette)) x Andrew

(Landhafer x (Mindo x Hajira-Joanette)) x Clinton

Vicland x (Landhafer x Richland-Bond)

Stem Infection 15% or Less

Garry x (((Forward x Victoria-Richland) x Vicland) x (Hawkeye x Victoria))

(Landhafer x (Bond-Rainbow x Hajira-Joanette)) x Andrew

(Landhafer x (Bond-Rainbow x Hajira-Joanette)) x Clinton

(Landhafer x (Mindo x Hajira-Joanette)) x Clinton

Landhafer x (Richland x Bond)

(Victory x Hajira) x (D69 x Bond)

DISCUSSION

The data indicate that wide differences in reaction to Septoria avenae exist among the presently grown oat varieties and among the new experimental selections.

Although there is a tendency for the later maturing oat strains to have the lowest disease scores (4), several of the earlier strains have consistently shown little Septoria infection under Wisconsin conditions. Thirty-four oat strains headed on June 26, 1957. An analysis of variance revealed a highly significant difference in reaction to Septoria among these lines. Strains with both high and low disease scores headed on the same day, indicating a varietal reaction to S. avenae which is independent of maturity (heading date).

Twenty-eight oat varieties were tested for 3 consecutive years. An analysis of variance of the stem infection data indicated a statistically significant variety-year interaction, but it was small in comparison to the variation among the varieties. Nine varieties had stem infection scores which deviated by more than 15 percent during the 3-year period, but only four of these deviated by more than 20 percent. The greatest deviation was 25 percent with two varieties.

The 361 oat strains were also grown and rated for reaction to Septoria at two other locations in the State in 1957. As they were exposed to natural infection only at these two locations, disease scores were lower; but the ranking of the oat strains was similar at all locations. Crown and stem rust infections were present in more than trace amounts at only one location in 1957, but these two diseases did not appear to greatly affect the Septoria reactions of the oat varieties or selections tested.

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THE RESISTANCE OF VARIOUS GRASSES TO OPHIOBOLUS GRAMINIS

David Gottlieb[1], Dora Volovsky de Hernandez[2], and Mario Rogers[2]

Chilean wheats have been troubled with "Mal de Pie" since the disease was first identified in Chile in 1940. The damage varies greatly, but severe epidemics occurred in 1941, 1947, 1952, and 1953. Recently, the isolation of Ophiobolus graminis Sacc. in pure culture has allowed more intensive study on this disease.

One line of investigation concerned the susceptibility of various grasses. This research was stimulated by the observation that the disease occurs even though a rotation is used in which a 2-year pasture is grown between wheat crops. The rotation cycle is longer when potatoes or oats are added to the cycle. Sometimes the pasture phase contained only grasses. At other times the pasture might be seeded to red clover alone, but even in this case wild grasses eventually become interspersed with or often replace the legume. Obviously, if grasses are attacked by the fungus the pathogen could be harbored in the soil, and when wheat was planted again, disease would result. The use of resistant species, on the other hand, could help eliminate the fungus from the soil.

A program for testing the reaction of various grasses was also vital from another viewpoint. Chile has begun to emphasize the need for greater acreage of pasture to increase milk and meat production. Any information on relative susceptibility to pathogenic fungi known to be present in the country would aid in the choice of grasses for pasture expansion.

The studies reported in this paper were carried out in the greenhouses of the Departamento de Producción Agraria, of the Ministry of Agriculture at Santiago. In all cases sterile soil in pots was overlaid with agar containing the mycelium of Ophiobolus graminis which was then covered with a layer of soil. Grass seed was sown over this inoculum. A very light layer of soil was placed over the seed and tamped gently. The results of two separate experiments are reported: a preliminary one in which duplicated pots of both infested and of non-infested soil were used for each grass but no wheat was used as a standard to determine the relative pathogenicity of the fungus, and a second trial in which wheat was used as standard. The 48 species in the trials were chosen either because of their importance as indigenous grasses or because they are currently being studied for possible incorporation into the forage program. The plants were removed from the pots after 6 weeks' growth, washed, and rated for degree of infection. Zero indicated disease-free roots; trace to 4, increasing amounts of necrosis; and 5, almost completely necrotic roots.

The data in Table 1 indicate that a wide variety of grasses are attacked by O. graminis under the drastic conditions of these experiments. Only 15 of the 47 species were entirely immune, although a number of others showed some degree of resistance. The species of Agropyron and Bromus tested were all very susceptible, whereas, at the other end of the scale, Agrostis, Arrhenatherum (oatgrass, listed under "fromental" in the Table), and Orzyopsis were resistant. In other genera, such as Lolium and Hordeum, the resistance varied with the species.

Because O. graminis is widespread through the Central Valley of Chile, the reaction of grasses to it is important. These data can act as a guide to further studies on the use of susceptible species in pasture rotations with wheat, where take-all occurs. Furthermore, even if rotation is not involved and permanent pasture is planned, the planting of resistant species would produce a better pasture. The data from the present experiment indicate that Agropyron and Bromus are generally susceptible. The use of these grasses on land infested with O. graminis should be carefully considered, because of the danger of poor vigor. Similarly, growing of Festuca rubra and Alopecurus pratensis should not be encouraged in infested areas. A sufficient number of resistant grasses are available, however, to allow a choice of good grasses for the different regions of Chile. Among the important resistant grasses are Dactylis glomerata, the three oatgrasses (fromental), Phalaris arundinacea, Festuca K31, and Sorghum vulgare. Other species of potential importance that are now being studied for their adaptability to Chilean conditions and are not attacked by O. graminis are Poa pratensis and the three species of Oryzopsis.

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Table 1. Reaction of a number of grass species to Ophiobolus graminis in greenhouse tests.

Species	Reaction	
	Test 1	Test 2
Agropyron (from El Volcan)	1	1,2
Agropyron intermedium P.F. 2236	5	3
A. elongatum	3	3
A. smithii P.F. 2232	3	3,4
A. trichophorum	4	4
Agrostis alba	0	0
A. tenuis	0	0
Alopecurus pratensis	3	0
Arrhenatherum elatius (see Fromental)		
Bromus macracanthus	5	5
B. arduenensis	4	3
B. carinatus	4	4
B. catharticus	4	4
B. inermis	5	5
B. unioloides	-	5
B. valdiviano	5	3
Chloris gayana	0	3
Cynodon dactylon	-	0
Cynosorus echinatus (from Nuble)	T	T
C. echinatus	0	0
Dactylis glomerata (Australia)	0	0-T
Eragrostis curvula	-	2
Festuca K 31	0	0
F. rubra	3	3
Fromental, common, P.I. 1523	0	0
Schroeder	0	0
Tualatin	0	0
Hordeum bulbosum	No germination	T-3
H. jubatum	No germination	0
H. murinum	No germination	3
Lolium sp. (resistant to rust) P.F. 2251	No germination	1
Lolium sp. (resistant to rust) P.F. 2252	4	T
L. perenne	-	4
L. perenne P.F. 2250	2	2-3
L. multiflorum (New Zealand)	1	0
L. multiflorum	3	T
Lolium sp. (L. multiflorum - L. perenne)	3	T
Oryzopsis coerulescens	3	T
O. hymenoides	0	0
O. miliacea	0	0
Paspalum dilatatum	0	0
Phalaris arundinacea	0	0
P. tuberosa var. stenoptera	0	0-2
Phleum pratense	0	0

Table 1. (Continued).

Species	Reaction	
	Test 1	Test 2
Poa pratensis	0	0
P. bulbosa	2	-
Sorghum vulgare	-	0
Triticum vulgare	-	4

A number of important grasses grown in Chile were susceptible in the greenhouse studies, and further investigations are important to determine the amount of damage that actually occurs under field conditions. Agropyron intermedium and A. elongatum are used extensively in dryland pasture in central Chile and could be badly damaged if the pathogen were present in those soils. These grasses are also used in the Magallanes region of southern Chile, where they grow well. Since no wheat is grown there, O. graminis might not be an important pathogen in the Magallanes region. Hordeum jubatum, which is also recommended for dryland pasture, was susceptible in the current experiments and may need further evaluation for disease reaction. Hordeum murinum, which is very widespread as a volunteer grass all through the agricultural regions, is only slightly susceptible and should not be an important reservoir for preserving inoculum of O. graminis in the soil between wheat crops. Another important weed grass, especially in southern Chile, is Holcus lanatus, which is often even sown as pasture in wheat rotations. Unfortunately, the seed available for our testing program was not viable, but H. lanatus has been reported to be susceptible to O. graminis and further studies on its reaction locally have been planned for the future.

A survey of the literature revealed that reports of the susceptibility of grasses to O. graminis varied (1 to 7). Generally, Agropyron and Bromus are susceptible; this is corroborated by the Chilean data. Studies in the United States indicated more resistance among the bromes than has been reported in other studies (5). Dactylis glomerata is reported susceptible in England, but not in Chile, France or the United States (3, 4, 5). Festuca rubra is susceptible in Chile but not in the United States (4). Hordeum jubatum and Sorghum vulgare are resistant in Chile but susceptible in other countries (3, 6). Poa pratensis is resistant in Chile and the United States but susceptible in England (4, 5). It would be wrong, therefore, to use the reaction of a grass to the strain of O. graminis present in one country as a guide to the value of grass in a different area.

When recommending a grass for pasture, many factors other than disease reaction are important, but certainly attack by a pathogen can severely limit the vigor of such crops and at times almost completely destroy them. Greenhouse data on susceptibility and resistance are not a sufficiently reliable indication of attack in the field to base the choice of crop entirely on the disease reaction under such artificial conditions. In root diseases, synergistic reactions among pathogens are very important. On the other hand, antagonisms might occur which prevent the active growth of the pathogen. Field studies, therefore, are necessary before a rational choice of pasture grasses for Chile is finally made.

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BURNING OF DORMANT DALLISGRASS SHOWS PROMISE OF CONTROLLING ERGOT CAUSED BY CLAVICEPS PASPALI STEV. & HALL[1]

Homer D. Wells, Glenn W. Burton, and James E. Jackson[2]

Abstract

Burning dormant Dallisgrass stubble seems to be effective for inactivating ergot sclerotia. This should aid in producing an early seed crop of Dallisgrass in the southeastern United States.

Ergot (Claviceps paspali Stev. & Hall) is an omnipresent disease of Dallisgrass, Paspalum dilatatum Poir., in the South. It is considered to be one of the major factors limiting commercial production of Dallisgrass seed in the United States. Observations at the Experiment Station, Tifton, Georgia, during 1954, 1955, and 1956 suggested that some Dallisgrass seed matures and can be harvested before the first ergot infection occurs. Ergot was first observed in the Dallisgrass seed blocks on June 25, June 28, and June 15 for the years 1954, 1955, and 1956, respectively.

In the spring of 1957, two fields of prostrate Dallisgrass approximately 2 miles apart were observed. Both fields had been badly diseased with ergot during the previous summer. Both fields were fertilized with 100 pounds of nitrogen per acre and adequate amounts of phosphorus and potassium during the latter part of March, 1957. The accumulated dead material was burned in one field in early March, 1957. The accumulated dead material in the other field was not burned or removed.

A routine survey on May 21, 1957, showed that the unburned field was already badly infected with ergot; whereas, no disease was present in the burned field. Numerous germinating sclerotia of Claviceps paspali (shown in Figure 1) covered the ground in the unburned field. In contrast, only one germinating sclerotium was found in the burned field during a 3-hour search.

Seed was harvested from the two fields on June 12 and 14. Data reported in Table 1 show that a 28.6 percent increase in seed yield was obtained in the burned field; however, the test was not replicated and it is questionable whether all of the increase can be attributed to the burning treatment.

Table 1. Effect of dormant burning on seed production and ergot control in prostrate Dallisgrass.

Treatment	Pounds of seed / acre	Floret content		
		Percent seed	Percent ergot	Percent empty florets
Burned March 1, 1957	90	59.5	3.6	34.4
Not Burned	70	51.0	14.6	36.9
F-value	--	4.97*	136.0**	1.89N.S.

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[1]Cooperative investigations at Tifton, Georgia, of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the University of Georgia College of Agriculture, Agricultural Experiment Stations. Published with the approval of the Director as Journal Series Paper Number 49.

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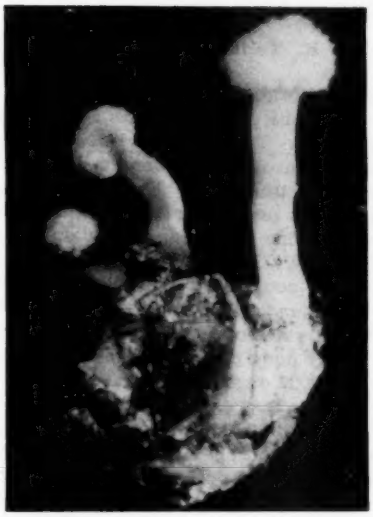

FIGURE 1. Germinating sclerotium of Claviceps paspali taken from ground in unburned field of prostrate Dallisgrass, showing heavy infection with ergot on May 24, 1957. About 15X.

Eight randomly selected samples of approximately 150 florets each were taken from the uncleaned seed harvested from each field. The florets were grouped according to content: whether they held seed, ergot, or were empty (Table 1). No significant difference was obtained in percentages of empty florets from the burned and unburned areas. The significant increase in percentage of seed in samples from the burned field in comparison with those from the unburned field suggests that some of the seed production potential was consumed or inhibited by the much greater ergot infection. These data suggest that burning of dormant Dallisgrass may be an effective way to control ergot and produce an early seed crop.

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OBSERVATIONS ON WHEAT STREAK MOSAIC IN WASHINGTON, 1955-1957[1]

G. W. Bruehl and H. H. Keifer[2]

Wheat streak mosaic has occurred each season following its discovery in Washington by Meiners and McKinney (3) in 1954. It was then known to be present on the experimental farms at Pullman, but did not occur in adjacent farmers' fields. Discovery of this disease on the experimental farms at Pullman and its absence in adjacent fields presented the unwelcome possibility that it could somehow have been introduced at this point. The observations of the succeeding years have allayed this fear, and indicated its prior existence in the State.

In addition to its regular occurrence at Pullman, it has been found in Adams, Asotin, Benton, Columbia, Garfield, Klickitat, Spokane, Walla Walla, and Whitman Counties at 15 separate points. In 11 collection points the mosaic was confined to one or a few plants, showing no ability to spread (Table 1). High infection was found at four points described below.

One high infection place is on the main experiment station at Pullman. Here, where the disease was first observed and where it persists, many poor cultural practices exist. Seeding date trials extend the period of green wheat abnormally through the summer. The station grows wheat-Agropyron hybrids with no real terminal maturity. These hybrids remain green through the summer and are winter hardy. They harbor the mite vector, Aceria tulipae K., and many of them are infected with mosaic. Horticulturists occasionally use spring-sown winter wheat as a ground cover. Thus, at Pullman we have had a constant source of infection.

In the second case of spread, 2 or 3 acres of thoroughly diseased winter wheat in Klickitat County, near Blockhouse, were sown on freshly plowed, stumpy, cut-over woodlot meadow. Much Canadian bluegrass, Poa compressa L., persisted in soddy tufts throughout the diseased area. This grass is a host to both the vector and the virus (4), and it could account for the survival of the mites through the year. Aceria tulipae was found on the Canadian bluegrass and on a weedy brome in this field (Table 2). This plot has been sown to wheat all three seasons. Mosaic has disappeared in it as the sod and weedy grasses were destroyed.

The third and largest infection was in a 180-acre field in Spokane County, 3 miles west of Deep Creek, that was practically 100 percent diseased. This field was so strikingly yellowed that it was readily detected in May 1957, from the highway while driving 50 miles per hour.

"Spring" wheat had been sown in this field in the spring of 1956, but the seed lot had contained about a 50 percent mixture of a Turkey-type winter wheat. When the spring wheat was harvested, the winter wheat remained as rosettes among the stubble. The Turkey type survived the summer and winter and was infected when seen the following May. Adjacent winter wheat sown at the normal time was essentially free of infection, showing that the diseased wheat had become infected the preceding summer. Mites were present in May 1957, and they were identified as Aceria tulipae (Table 2).

In a fourth observation of extended infection, about 40 acres of abnormally late-sown spring wheat in Garfield County were generally infected. An adjacent field containing old volunteer wheat had just been plowed. It probably had contained infected plants, which served as the source of inoculum.

These field observations of failure to spread in most cases and active spread in four may be explained on the bases of the studies of epidemiology by Slykhuis (4) and Staples and Allington (5). The main point emphasized in their studies is that the mite has no difficulty overwintering on winter wheat, but that the time of stress is the hot, dry period of the summer. Accepting this, we are able to conjecture as to the failure of wheat streak mosaic to spread to any extent in this State during the past 3 years, even though the virus and the vector were both present.

Staples and Allington (5) found that volunteer wheat must be infested early in the summer to build up a high mite population and spread the virus to any extent. All four cases of active spread in Washington developed where young green host tissues were infested during the warm months. Eriophyid mites are associated with soft, usually green tissue, and depend upon

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[1]Scientific paper No. 1676, Washington Agricultural Experiment Stations, Pullman; work conducted under Project No. 1280, and published with the approval of the Director, California State Department of Agriculture.

[2]Associate Pathologist, Washington State College, and Systematic Entomologist, California Department of Agriculture, respectively. The senior author assumes responsibility for the field work and interpretations, the junior author is responsible for all identification of mites.

Table 1. Localities of observed occurrence of wheat streak mosaic in Washington 1955-1957.

PLACE	REMARKS
Adams County	
Lind	Volunteer wheat along road, summer, 1955. Not seen in area since.
Asotin County	
Anatone	A single plant, regular seeding, in outer drill row along road, summer, 1957.
Benton County	
Prosser	A few plants, remnants of winter wheat cover crop among irrigated hops (first seen by J. W. Hendrix). Summer, 1955.
Columbia County	
Dayton	Summer, 1955, a single volunteer plant in a bottomland field. Summer, 1957, a few volunteer plants in high fields south of town.
Long	Summer, 1956, several volunteer plants along the ditches bringing irrigation water to asparagus fields.
Garfield County	
Pomeroy	About 40 acres of very late-sown spring wheat heavily infected. Volunteer wheat in adjacent field. Summer, 1957.
Klickitat County	
Bickleton-Cleveland area	A few individual plants, both volunteer and regular seeding, in all three seasons.
Blockhouse	Two or three acres generally infected in 1955. Infested with *Poa compressa*, the probable source of infection. In 1956 this field was freer of grassy weeds and had only a few infected wheat plants. Fall, 1957, apparently clean.
Spokane County	
Deep Creek	180 acres of winter wheat thoroughly infected. The remnant of a mixed seeding of spring and winter wheat of the previous season.
Walla Walla County	
Walla Walla	A few winter wheat plants of the regular seeding, summer 1955, along a drainage ditch in which several grass species were growing.
Whitman County	
Pullman	Main Experiment Station. Present each year since its discovery in 1954.
Dusty-Lacrosse area.	1955-volunteer plants of winter wheat--no spread to regular seeding drilled right over them. 1955-1956 diseased volunteer plants along the roadside. No spread into adjacent fields.

Table 2. Collections in Washington of three eriophyid mites on grasses.

Mite	Host	Locality	Date
Aceria tulipae K.	Triticum aestivum L.	LaCrosse	Oct. 18, 1955
	" "	Pullman	Dec. 27, 1955
	" "	Pullman	June 12, 1956
	" "	Long	Apr. 17, 1956
	" "	Deep Creek	May 17, 1957
	" "	Prosser	Nov. 11, 1955
	Bromus inermis Leyss.	Pullman	Nov. 10, 1955
	B. marginatus Nees	Pullman	Nov. 4, 1955
	B. sp.	Pullman	Nov. 3, 1955
	B. sp.	Blockhouse	Apr. 17, 1956
	Poa compressa L.	Blockhouse	Apr. 17, 1956
Vasates mckenziei K.	Triticum aestivum L.	Pullman	Nov. 3, 1955
	Bromus catharticus Vahl	Pullman	Nov. 3, 1955
	Elymus junceus Fisch.	Pullman	Nov. 3, 1955
	Festuca elatior L.	Pullman	Nov. 10, 1955
Abacarus hystrix Nal.	Aegilops cylindrica	Pullman	June 12, 1956

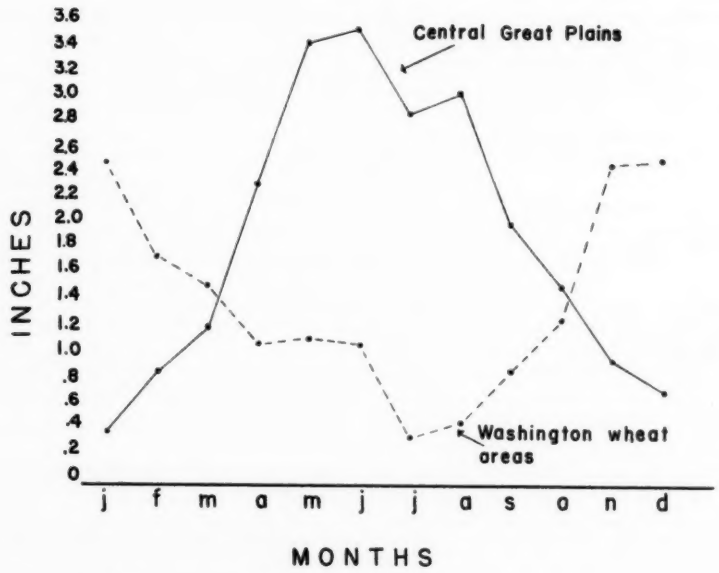

FIGURE 1. Monthly precipitation in the central Great Plains and Washington wheat areas. The central Great Plains data curve is the average of Hayes, Nebraska, and Russell and Hoxie, Kansas. The Washington wheat curve is the average of Davenport, Goldendale, and Pullman. Data taken from Climate and Man, United States Department of Agriculture Yearbook, 1941.

large populations to insure distribution (1). The normal climate of the major Washington wheat areas and present cultural practices combine to prevent mite build-up.

Summers in our wheat areas are almost lacking in rainfall, which results in a dearth of succulent tissues to harbor the mite (Fig. 1). Most farmers have clean fallow with little or no volunteer wheat or grassy weeds. Staples and Allington stressed hail as important in establishing volunteer wheat and prolonging the vegetative stage of the crop (5). Similar observations in Wyoming (6) and Montana (2) confirmed the importance of hail. Hail is infrequent in Washington and moisture is so limited during the summer that very little wheat volunteers. This dearth of green wheat and grasses reduces the mite population to traces surviving in occasional favored locations. Buildup with the fall rains has not been sufficient to spread the disease. Even though it is possible that some natural enemies or disease act to suppress mite populations, climatological data seem to provide an adequate explanation, especially when the clean fallow typical of the area is considered.

Three Eriophyid mites have been found on wheat and associated grasses in Washington: Aceria tulipae K., Vasates mckenziei K., and Abacarus hystrix (Nal.). The first mite is coextensive with our mosaic infections. Also, it has transmitted the virus to healthy wheat seedlings grown in the greenhouse. There is no evidence to implicate the other two mites as vectors. Most of the mite specimens collected in these studies have had relatively few individuals present.

The erratic appearance of this disease in Washington over a wide area indicates the existence of an unknown grass host (or hosts) where the virus and the mite persist in trace amounts. So far this disease has been of minor importance, but its existence in the State poses a constant threat. The ability of the mite to "come from nowhere" and thoroughly infest and infect 180 acres of wheat, as in Spokane County in 1957, illustrates the potential danger from wheat streak mosaic in Washington.

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BARLEY YELLOW DWARF VIRUS DISEASE OF OATS IN NEW YORK[1]

W. F. Rochow[2]

Summary

The barley yellow dwarf virus disease of spring oats is as widespread in New York as in other areas that have been studied. At least a few infected plants were found in each of 122 fields inspected over a 2-year period. A detached-half-leaf method was used to compare virus transmission from infected field plants by means of apple grain and English grain aphids. Thirty-two samples from 10 counties were tested by this method. Virus was recovered from 26 of these samples by English grain aphids only, from two samples by apple grain aphids only, and from one sample by both aphids. Preliminary studies with some of the virus isolates indicated that these results reflect great differences in vector efficiency and not necessarily absolute specificity.

Although barley yellow dwarf virus disease of oats has been reported from New York (5), little is known about the vectors of the causal virus or about the distribution of the disease in this area of the United States. During the growing seasons of 1956 and 1957, surveys were made to determine whether the disease was as widespread in this region as in other areas of the country that have been studied (2, 5). In connection with these surveys, attempts were made to confirm the diagnosis of the disease by transmission of the virus by means of aphids. In 1956, relatively few of these transmissions were successful when tests were made with apple grain aphids in a manner used successfully by other workers (1, 5) or by means of the detached-leaf method described below. In 1957, special tests were made to study the possibility that the apple grain aphid is not an efficient vector of many of the isolates of the virus that occur in New York. This paper describes the results of the tests carried out in 1957 as well as the technique that was used to compare transmission by apple grain and English grain aphids.

SURVEY

Symptoms of the disease in New York were similar to those described by others (2, 3, 5). Although the red-leaf symptom was observed in some fields late in each season, this symptom was not a common expression of the disease.

In 1956, 50 fields of spring oats in 15 counties of New York were inspected. At least several infected plants were found in every field. In seven of the fields an estimated 20 to 30 percent of the plants were infected, and in a few fields about 50 percent of the plants had symptoms typical of the disease.

In 1957, 72 fields of spring oats in 17 counties were examined. Infected plants were found in every one of these fields, but infection was present only in trace amounts in most cases. On the basis of these surveys, the barley yellow dwarf virus disease of oats appears to be as widespread in New York as in other areas that have been investigated (2, 5).

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GENERAL MATERIALS AND METHODS

All stock colonies of virus-free apple grain (AG) aphids (Rhopalosiphum fitchii (Sand)) and English grain (EG) aphids (Macrosiphum granarium (Kirby)) were maintained on caged plants in a laboratory to isolate them from plants infected by barley yellow dwarf virus (BYDV). AG aphids originally were supplied by R. M. Endo. EG aphids were collected in the field near Ithaca, New York, in the fall of 1956. New colonies were started several times each week. Viviparous females were placed on an oat seedling in a Petri dish containing moist filter paper, and newly emerged nymphs were transferred to seedlings of Dubois oats or of Erie barley in about the two-leaf stage. Seedlings were grown in 4- or 6-inch pots with about five plants per pot. After the plants were infested with about five to ten nymphs, the plants were covered with pot cages made from Pyralin cellulose nitrate plastic and Lumite saran screen. Pots were placed either on a window sill or under continuous fluorescent illumination. When sufficient aphids had developed in a colony, the aphids were used and the plants were discarded. Although every colony maintained in this way has remained nonviruliferous, separate controls were included in each test.

The reference strain of BYDV used in these studies was a moderately severe isolate supplied by R. M. Endo. Hereafter it will be referred to as the Endo strain of BYDV.

DETACHED-LEAF METHOD

Since it was found that aphids fed well on detached cereal leaves in humidified Petri dishes, the possibility of using this method to test field material was investigated. The procedure described below finally was adopted.

Oat leaves with symptoms of the BYDV disease were collected in the field and placed in plastic bags, which usually were stored over ice. Only one leaf was collected from a diseased plant. The leaves were taken to a laboratory where about a 5-inch portion was cut from the center of each leaf to be tested. In some cases, this entire portion was used; in other cases, the leaf section was cut in half longitudinally and opposite halves were placed in separate dishes so that one could be infested with AG aphids and the other with EG aphids. The leaf section was folded so that it rested on edge in the dish to allow aphids to feed on both surfaces. A piece of filter paper in each dish was moistened with distilled water or with a solution of 10 mg/liter of the plant growth regulator, kinetin (4). Kinetin sometimes lengthens the period during which detached oat leaves remain green, but this was of no known importance in the work reported here. Each leaf section was then infested with about 40 to 50 nonviruliferous AG or EG aphids and stored in the laboratory to allow an acquisition feeding period of 24 to 48 hours. A comparable group of aphids from each colony used was placed in a separate Petri dish on a healthy seedling to serve as a nonviruliferous control. Sometimes aphids also were colonized on leaves from Dubois oats infected with the Endo strain of BYDV to serve as a viruliferous control.

At the end of the acquisition feeding period, the Petri dishes were taken to the greenhouse where the aphids were transferred to test seedlings by means of a camel's-hair brush. Aphids from each dish were transferred in groups of about ten per seedling to two to three test seedlings that were grown in steam sterilized soil in a 4-inch pot. Most of the aphids used were mature apterous females, but other forms often were included in each group. Test seedlings were caged with pot cages and maintained on a separate greenhouse bench during a 3-day test feeding. In most cases, Coast Black oats (C.I. No. 1025) were used as test plants; Garry oats (C.I. No. 6662) or Atlas 46 barley (C.I. No. 7323) were used in some cases. After the 3-day test feeding period, all aphids were removed by fumigation with Lindane in a closed chamber. The plants were placed on a greenhouse bench under supplemental illumination and were observed at intervals for at least 4 weeks. Symptoms usually appear after 10 to 14 days, but in some cases symptoms did not appear until about 3 weeks after the test feeding.

The reliability of this method was determined in tests with AG aphids and the Endo strain of BYDV. Leaves were removed from infected oat or barley plants in the greenhouse, placed in Petri dishes, and infested with nonviruliferous AG aphids as described above. Healthy seedlings of oats or barley were infested as controls.

In 11 experiments, transmission of the virus occurred in every case in each experiment when groups of about 10 AG aphids per test seedling were used. In one test with two aphids per seedling, nine of the 11 test plants became infected. In two experiments with five aphids per test seedling, a total of 14 of 16 test plants became infected.

To test the importance of the time interval between removal of the leaf from the infected plant and infestation with aphids in the Petri dish, detached leaves were stored for various times before use. Virus was recovered in all attempts when detached oat leaves were stored for 1 or 2 days at room temperature in Petri dishes with moist filter paper or when leaves were kept for as long as 4 weeks at 3° C in plastic bags before they were infested with nonviruliferous AG aphids (Table 1). A similar experiment with infected barley leaves gave the same results as those shown for oats in Table 1.

Table 1. Transmission by apple grain aphids of barley yellow dwarf virus (Endo strain) from detached oat leaves stored for various intervals.

Test	Storage		No. of leaves	No. of test plants[c] infected when source leaves were as indicated	
	Days[a]	Temperature[b]		Infected	Healthy
1	None	--	1	2/2	0/3
2	1	RT	1	3/3	0/3
	1	3° C.	1	3/3	-
3	2	RT	1	3/3	0/3
	2	3° C.	1	3/3	-
4	15	3° C.	4	12/12	0/3
5	28	3° C.	4	8/8	0/3

[a] Time between removal of leaves from infected plants and infestation with nonviruliferous apple grain aphids in Petri dishes.

[b] RT indicates room temperature (about 21° - 30° C.)

[c] Numerator is the number of Coast Black oat seedlings that became infected; denominator is the number of seedlings that were infested with about 10 aphids.

RESULTS

Thirty-two oat plants with symptoms of the BYDV disease from ten counties of New York were tested by means of the detached-half-leaf method. BYDV was recovered from all but three of these samples (Table 2). Virus was recovered from 26 samples by EG aphids only, from two samples by AG aphids only, and from one sample by both aphids. In each test the nonviruliferous controls were uniformly negative.

In addition, another 36 infected field plants representing collections from six counties were tested only by means of AG aphids on a total of 144 test plants. Virus was recovered from only four of these samples.

Since the AG aphid generally has been considered to be the best vector of BYDV and has been used almost exclusively by some workers, this apparent specificity was investigated further.

One possible explanation for the failure of AG aphids to transmit BYDV from most of the New York field material might be based on some peculiarity of the particular AG colony used. Since the colony used here never failed to transmit the Endo strain of BYDV in parallel tests, however, there was no indication of any change in the colony. A fresh collection of AG aphids was obtained from R. M. Endo in Illinois, and another colony was started from AG aphids collected in the field near Ithaca, New York. These two new colonies then were compared with the regular colony in tests on leaves from infected field plants. Leaves were handled as described above except that each leaf was cut into 3 longitudinal sections so that it could be tested with aphids from each of the three AG colonies. No evidence of any differ-

Table 2. Summary of results of barley yellow dwarf virus transmission from opposite half-leaves of oats by means of apple grain (AG) and English grain (EG) aphids.

County where leaves were collected	No. of plants tested	No. of plants from which virus was transmitted by indicated aphid			No. of test plants[a] infected following tests with indicated aphid	
		AG	EG	Both	AG	EG
Broome	4	1	3	0	2/11	6/8
Madison	1	0	0	0	0/8	0/2
Tompkins	14	1	11	1	3/40	23/29
St. Lawrence	2	0	2	0	0/5	3/3
Jefferson	1	0	1	0	0/3	1/1
Seneca	2	0	1	0	0/4	3/6
Ontario	2	0	2	0	0/4	6/6
Monroe	2	0	2	0	0/4	6/6
Livingston	2	0	2	0	0/3	6/6
Steuben	2	0	2	0	0/4	6/6
Totals	32	2	26	1	5/86	60/73

[a] Numerator is number of Coast Black oat test seedlings that became infected; denominator is the number of seedlings that were infested with about 10 aphids.

ence among the three colonies was obtained. In one experiment, for instance, one leaf was tested from each of six oat plants with typical symptoms of the BYDV disease. No virus was recovered from five of these leaves by aphids from any of the three colonies, and virus was recovered from one leaf by aphids from all three of the AG colonies. There appears to be no reason to suspect any anomaly in the AG colony used in these studies.

A second possible explanation for the failure of AG aphids to transmit virus from most of the field collections is the presence of some type of vector-specific strains of BYDV in this area. Such strains have recently been reported from Washington (7), although their occurrence apparently is rare in that region.

Twenty-three subtransfers were made from 11 different virus isolates that originally were transmitted only by EG aphids (Table 2). Both AG and EG aphids were used in these tests in which the detached-half-leaf method was utilized. EG aphids transmitted virus to Coast Black oats in every case but one. Fifty-six of 59 test plants became infected. In contrast, AG aphids transmitted the virus only to one of 61 test plants. In parallel tests with the Endo strain of BYDV, AG aphids from the same colonies transmitted the virus in every case.

As a further test of this apparent specificity, two plants of Coast Black oats, each infected with a different isolate of "EG-specific" virus, were infested with nonviruliferous AG aphids. After 3 weeks, during which colonies developed, AG aphids were transferred from each of the two plants (in groups of about ten) to each of 15 seedlings of Coast Black oats, to 12 seedlings of Garry oats, and to three seedlings of Atlas 46 barley. AG aphids from one of the source plants transmitted virus to four plants of Coast Black oats, and to three plants of Garry oats. Aphids from the other plant transmitted BYDV to one plant of Coast Black oats and to one plant of Garry oats. Of the total of 60 test plants used in this experiment, then, only nine plants (or 15 percent of the test plants) became infected. Under comparable conditions with other virus isolates, AG aphids regularly effect 100 percent transmission of BYDV. It is clear that AG aphids do not transmit all isolates of BYDV with equal efficiency.

From two of the 32 field samples tested with both aphids, virus was transmitted only by AG aphids (Table 2). Five subtransfers were made from these two isolates by means of the detached-half-leaf method. Virus was transmitted to all 12 test plants by AG aphids, but none of the 11 test plants infested with EG aphids became infected. When nonviruliferous EG aphids

were reared on a plant infected with one of the isolates, however, they transmitted BYDV to six of nine test plants. It may be that EG aphids transmit these two isolates reasonably well only when the acquisition feeding period is greater than 48 hours.

The failure of EG aphids to transmit these two isolates from detached leaves is not particularly surprising, since EG aphids frequently are less efficient than AG aphids as vectors of BYDV. In a comparable series of tests with the detached-leaf method, for example, AG aphids transmitted the Endo strain of BYDV to all 18 test plants, but EG aphids transmitted the virus only to eight of 16 plants. These results are in general agreement with reported relative efficiencies of the two aphids as vectors of the Washington strain of BYDV (6).

DISCUSSION

These results differ from those of Toko and Bruehl (7) in that specificity was rare in Washington, whereas "vector-specific" isolates appear to be common enough in New York to be of possible importance in the natural occurrence of the disease. It is not yet clear, however, just what type of specificity is involved. At least two general interpretations might be given to these results.

Firstly, some of the isolates may be strains of BYDV that are vector-specific in an absolute sense. AG aphids failed in many tests to transmit certain isolates under conditions that regularly have resulted in transmission of other isolates of BYDV to every test plant used. In this work, more than 200 plants were used in various tests of transmission of "EG-specific" BYDV by AG aphids. Very few of these transmissions were successful. The one transmission of "EG-specific" BYDV by AG aphids in the first subtransfer of 11 of the isolates may have resulted from contamination. In this view, the transmissions that occurred after AG aphids were reared on plants infected with "EG-specific" isolates might be explained by assuming that the long acquisition feeding period increased the chance for AG aphids to acquire a mutant or one strain from a mixture.

Secondly, the data might be interpreted as a reflection of relative efficiency of the vectors. Perhaps the failure of AG aphids to transmit "EG-specific" virus from detached leaves was due to an inadequate acquisition feeding time. This period was never more than 48 hours in the tests that failed, whereas it was 3 weeks in tests where some transmissions did occur. This hypothesis appears to be the more likely one for the two cases of apparent "AG-specific" isolates, since one of these isolates has been transmitted with reasonable efficiency after a long acquisition feeding period. Moreover, EG aphids are known to be less efficient than AG aphids in the transmission of some strains of BYDV (6). Further tests with some of the isolates may clarify the interpretation of these data.

In connection with explanations for the occurrence of the BYDV disease in nature, these results emphasize the need for a better understanding of the aphid populations that occur on small grains. Isolation of "vector-specific" strains may depend on the time of the season when samples are collected in relation to seasonal development of different aphids. In the present study, most of the successful transmissions by AG aphids were from samples collected late in the season.

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ASTER YELLOWS AND CURLY TOP ON VEGETABLES IN NEW MEXICO

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Increased acreage of vegetables in southern New Mexico has focused attention upon many new production problems.

Observations in lettuce and spinach plantings have shown that two virus diseases, aster yellows and curly top, are serious production hazards.

Aster Yellows: In a lettuce variety and planting date test, conducted at the New Mexico College of Agriculture and Mechanic Arts, three dates of planting were as follows: July 23, August 2, and August 13. Aster yellows symptoms in the July planting were first noted on August 28. Counts made just before harvest showed that an average of 55 percent diseased plants were present in the July 23 planting, while in the other two plantings only 1 to 2 percent of the plants were diseased. The plots were dusted on August 21 with Malathion and on August 29, September 12, and September 19 with Parathion and DDT. Apparently the leafhopper invasion from native vegetation was at its height in late July. Table 1 below shows the varietal reaction to aster yellows in the July 23 planting.

Table 1. Tolerance of lettuce varieties to aster yellows.

Variety	Percent diseased or dead plants	Variety	Percent diseased or dead plants
Great Lakes Gem	62	Great Lakes #66	55
Great Lakes Premier	49	Great Lakes #118	49
Great Lakes Regular	55	Great Lakes 407-P	43
Great Lakes Shippers Strain	53	Great Lakes R-200	52
Great Lakes #13	38	Great Lakes 659	51
Great Lakes #54	40	Imp. #749	47
Great Lakes #59	47	A-36	63

The aster yellows incidence ranged from 38 to 63 percent. From a commercial standpoint, the low reading of 38 percent for Great Lakes 13 does not indicate sufficient tolerance to safeguard against disease losses.

A survey of the commercial plantings made before August 1 in southern New Mexico showed considerable losses to aster yellows. Some early-planted fields were abandoned because of the disease. Plantings made after August 1 did not show more than 1 to 2 percent loss from the disease at harvest time. One year's results indicate that serious losses from aster yellows can be averted by planting after August 1 in southern New Mexico. July planting, to allow for earlier harvest, does not seem practicable.

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Curly Top: Curly top observations were also made in two spinach variety tests planted on August 22 and 27, 1957. The disease was so severe that the variety tests were abandoned. The same was true of the 1956 spinach variety tests planted on August 15 and September 5.

A very low percentage of the healthy plants was present on October 19, 1957, when the disease counts were made. Table 2 lists the varieties and their reaction to curly top.

Table 2. Tolerance of spinach varieties to curly top.

Variety	Percent diseased or dead
Viroflay	71
Bloomsdale Savoy Long Standing	77
Giant Nobel	85
America	88
Hybrid #7	94
Bloomsdale Long Standing Re-Selected	97

Two years' testing has indicated that the severity of curly top probably precludes the planting of spinach in late August in the Mesilla Valley of southern New Mexico. Tests will be conducted again in 1958.

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POSSIBLE RELATION BETWEEN HAYWIRE OF POTATO AND BIG BUD OF TOMATO

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The "haywire" disease of potato (Fig. 1), which is caused by a virus, is endemic to a number of seed-producing States including Colorado, Idaho, Nebraska, Utah, and Wyoming. The disease has been observed also in some Southern States in potato plants grown from seed-stocks originating in the Western States listed, particularly in Nebraska.

"Haywire" is not a serious disease of the potato. However, it has been shown to be one of the many factors contributing to the spindling-sprout disease complex of seed potatoes. In connection with the investigation of the relation of haywire to the spindling-sprout disease, tubers from haywire-infected plants of the Dazoc variety were received from Marx Kochnke, Head, Nebraska Seed Certification Service, in 1954. In the course of the investigations the virus was introduced into tomato plants. These plants later developed symptoms similar to those of tomato "big bud" described by Samuel et al. (5) in Australia and by Dana (3) in the Pacific Northwest.

Natural infection of potato by the tomato big bud virus was first reported from Australia, where the disease produced is known as purple top wilt (1). In 1951, this disease caused serious losses in the varieties Sebago and Sequoia in New South Wales (2). Although haywire has not yet become a limiting factor in potato production in the United States, it nevertheless was considered advisable to conduct further studies to determine its relation to tomato big bud.

The virus causing haywire was transferred by approach grafting diseased plants of Dazoc potato to healthy plants of potato seedling 41956 (immune from virus X) to eliminate virus X from the disease complex. Diseased plants of seedling 41956 were than approach-grafted to healthy plants of Dazoc and Katahdin and to tomato plants of the varieties Marglobe and Rutgers. Twenty-nine days after grafting, the potato plants developed apical stunting, leaf rolling, and leaf pigmentation typical of haywire. The first symptoms on inoculated tomato plants of both varieties developed in 30 days. The apical meristems almost ceased elongating. Newly formed leaves and leaflets developed a yellowish-green color and did not expand normally. Affected leaflets rolled downward along the midrib and the leaflet tips became recurved. Fruits from fully opened flowers on affected plants ceased to develop and turned whitish green, and the ovaries of some cracked open. Unopened or partially opened flowers ceased development.

As affected plants grew older, the main stems slowly enlarged (Fig. 2). Newly formed leaves and leaflets were very small and malformed. Petioles and older leaflets showed severe epinasty, and most of the leaflets showed a purplish pigmentation, particularly on the lower surface.

The youngest fruit truss on diseased plants assumed an erect position (Fig. 2) and did not become recurved as in normal plants. The buds on each truss also remained in an upright position. The calyx on each bud remained united almost to the tip and gradually enlarged to a bladder-like form (Fig. 2); this is the most pronounced early symptom of big bud (5). These buds failed to open during the life of the plants, and phyllody was not observed. The latter may have been prevented by the high greenhouse temperatures toward the end of the test.

A pruned tomato plant infected with the haywire virus is shown in Figure 3. Lateral shoots developed from most leaf axils, and these produced dwarfed and yellowish-green leaves. These shoots were short and enlarged in diameter, reaching a diameter similar to that of the main stem. Numerous adventitious root initials developed on the enlarged stems of older affected plants.

When cross sections of the enlarged stems were examined macroscopically, a thin ring of watersoaked tissue was clearly visible on the inside of the vascular ring.

Stem sections from diseased tomato plants were approach-grafted to healthy plants of seedling 41956, Dazoc and Katahdin potatoes. These plants developed symptoms identical with those induced by grafting the original virus source plants.

In view of the observations and experiments made in Utah and comparisons of published accounts of haywire and curly top, Gardiner (4) suggested that haywire was caused by the curly top virus. The present authors found no similarity in symptoms induced in tomato (Fig. 4) by the curly top virus infecting tomato and potato plants in the vicinity of Harlingen, Texas,

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FIGURE 1. Haywire-infected plant of Triumph potato.

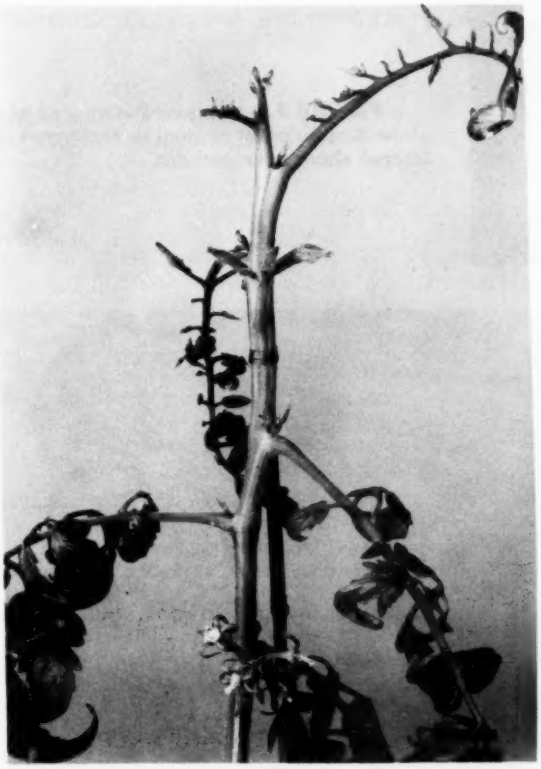

FIGURE 2. Rutgers tomato plant 50 days after approach-grafting with haywire-infected potato plants.

FIGURE 3. A haywire-diseased Marglobe tomato plant pruned to show type of lateral shoot development.

FIGURE 4. Rutgers t
mato plants: left, infected
with the curly top virus;
right, healthy.

and those induced by the haywire virus from Nebraska. Instead, their results indicate that haywire of potato in Nebraska may be caused by the tomato big bud virus.

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THE DEVELOPMENT AND EVALUATION OF RESISTANT PEPPER VARIETIES AND SELECTIONS BY MEANS OF ARTIFICIAL INOCULATION IN THE FIELD WITH TOBACCO MOSAIC VIRUS[1]

D. F. Crossan and E. M. Rahn[2]

Abstract

Artificial inoculation of several pepper varieties in the field with tobacco mosaic virus resulted in drastic reduction of yield of a susceptible variety. Three resistant varieties or selections were considered superior to California Wonder, with respect to yield, uniformity of plants, fruit shape, and wall thickness. The latter two characteristics are particularly desirable in canning-house peppers.

A survey of pepper (Capsicum frutescens L. var. grossum Bailey) fields in Delaware in 1944 by Heuberger (3) and in 1950 by Comegys (1) revealed that tobacco mosaic was common and prevalent in commercial plantings. Comegys (2) also studied the influence of tobacco mosaic virus (TMV), alone and in combination with other viruses, on yield of pepper fruit. He found that TMV drastically reduced marketable yields.

In 1952 a search was begun to obtain a variety or selection with resistance to TMV that also possessed the thick-walled, blocky fruit desired by canners.

Dr. C. N. Haenseler, New Jersey Agricultural Experiment Station, provided pepper seed from plants with suspected resistance, which had first originated with Dr. F. O. Holmes of the Rockefeller Institute for Plant Research, Princeton, New Jersey. Field plantings were made in cooperation with the H. P. Cannon & Son Company[3], Bridgeville, Delaware, by Comegys in 1950-1953 and by the senior author in the period 1954-1956. Field selections were made from plants with promising fruit type and large leaf size for fruit shading.

In 1954, seedlings of two of the more promising selections, tentatively named Caldel and Calcom by the H. P. Cannon and Son Company, were raised in the greenhouse and artificially inoculated with TMV when the plants were in the four- to five-leaf stage of growth. In the Calcom selection, approximately 50 percent of the seedlings were susceptible; in the Caldel selection, approximately 75 percent of the seedlings showed the leaf abscission-type resistance and about 25 percent exhibited the systemic necrosis-type resistance (4). Resistant plants were screened and self-pollinated. This procedure was repeated for three generations. Seedlings of the third selfed generation were planted in isolated half-acre blocks and each plant inoculated by rubbing two leaves at random with a suspension of TMV and carborundum. Three weeks after inoculation suspected plants were rogued. Individual plant selections were made later on the basis of resistance, plant characteristics, and fruit type.

In 1957, at the University Substation, Georgetown, Delaware, a joint test was conducted by the Departments of Plant Pathology and Horticulture, comparing a TMV-susceptible variety (inoculated and uninoculated), several commercial varieties of known resistance, and the Caldel and Calcom selections for yield and other characteristics.

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[3]The authors wish to express their appreciation for the complete cooperation of the H. P. Cannon & Son Company in providing land, plants, and labor and in aiding in the selection of desirable pepper lines.

Table 1. Number of pepper plants showing symptoms of tobacco mosaic one month after inoculation with the virus[a].

Variety or selection	Category	Number of plants with symptoms: Replicates				Average
California Wonder	Inoculated	19	18	24	24	21.2
California Wonder[b]	Uninoculated	1	5	5	4	3.7
Yolo Wonder A	Inoculated	0	0	0	0	0.0
Improved Yolo	Inoculated	5	1	4	2	3.0
Keystone Resistant Giant	Inoculated	0	0	0	0	0.0
Calcom	Inoculated	4	3	2	5	3.5
Caldel	Inoculated	0	0	0	0	0.0

[a]Average of 24 plants per replicate.
[b]Numbers represent natural field infection.

Table 2. Comparison of yield, fruit shape, wall type, and maturity of marketable fruit from TMV-susceptible and resistant pepper varieties or selections.

Variety or selection	Yield[a] (lbs.-10ths)	Fruit shape (1-5)[b]	Wall type (1-5)[c]	Maturity peak
California Wonder inoculated	8.4	4.5	3.8	Midseason
California Wonder uninoculated	47.8	2.5	2.4	Midseason
Yolo A inoculated	73.4	2.0	2.6	Midseason
Improved Yolo inoculated	59.0	2.0	2.6	Midseason
Keystone Resistant Giant inoculated	74.5	2.1	2.5	Midseason
Calcom inoculated	71.8	2.5	3.2	Late season
Caldel inoculated	79.4	3.2	2.7	Late season
L.S.D. .05 = 7.3				
L.S.D. .01 = 10.0				

[a]Average yield of 4 replicates.
[b]Relative scale where 1 = large, blocky, uniform bell-type and 5 = small, variable sized and shaped fruit.
[c]Relative scale where 1 = wall thickness greater than approximately 3/8 inch and 5 = wall thickness of about 1/8 inch.

EXPERIMENTATION AND RESULTS

The California Wonder variety (uninoculated and inoculated) was used as the standard of comparison for the varieties Yolo Wonder A, Improved Yolo, Keystone Resistant Giant, and the Calcom and Caldel selections.

The varieties and selections were planted in a randomized, block design of four replications. Forty days after planting, the plants were inoculated by rubbing two leaves at random on each plant with a cheesecloth pad saturated with a suspension of TMV. Counts were made 1 month later to determine the number of infected plants (Table 1). The fruit were picked five times between July 25 and September 6, 1957, and graded into marketable and cull categories according to canning standards for size and red color. The data were analyzed statistically and an L.S.D. value obtained for comparison of any two means (Table 2). Notes were taken on maturity, yield, fruit shape and wall thickness (Table 2).

DISCUSSION

It is evident from the data obtained that tobacco mosaic drastically reduced the marketable yield of fruit in the susceptible variety California Wonder. The damage consisted of reduction in total number of fruit formed, fruit malformation, and irregular coloration. The varieties Yolo Wonder A, Keystone Resistant Giant, and the Caldel selection were highly resistant to infection by TMV and represented pure lines in this respect, in this test; Improved Yolo and Calcom contained a high percentage of resistant plants but were not pure lines. Keystone Resistant Giant and Caldel were considered to be outstanding with respect to resistance, uniformity of plants, yield, shape of fruit, and thickness of fruit wall, the latter two characteristics being particularly desirable to growers of canning-house peppers. Keystone Resistant Giant produced an earlier and heavier set of fruit initially over Caldel but by the latter part of the season Caldel had set and matured an equally heavy yield of fruit. Caldel had the added advantage of possessing very large, broad leaves which aid materially in reducing damage from sunscald. Both Keystone Resistant Giant and Caldel are worthy of consideration by canners or fresh market growers in the Del-Mar-Va area.

Yolo Wonder A was equal to Keystone Resistant Giant and Caldel in most respects, but it had a tendency to produce a number of strongly tapered fruit rather than the more characteristic blocky, bell type. Nevertheless, in spite of this tendency, it was still a highly desirable pepper.

Improved Yolo and Calcom were highly variable with respect to fruit type, and contained a number of susceptible plants. This was strongly reflected in the yield of Improved Yolo but not in that of Calcom.

Natural infection by TMV in the uninoculated California Wonder plants resulted in a drastic reduction in yield as compared with resistant varieties, but the comparison with inoculated California Wonder was even more striking. In general, plant uniformity, yield, fruit shape, and wall thickness of California Wonder were considered inferior to these characteristics in the resistant varieties or selections tested.

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DELAWARE AGRICULTURAL EXPERIMENT STATION

THE HOSTS AND STRAINS OF PEA ENATION MOSAIC VIRUS[1]

Frank P. McWhorter[2] and W. C. Cook[3]

Abstract

Observations made in the Milton, Oregon area indicate that alfalfa, Medicago sativa L., is probably the principal local source of pea enation mosaic virus. These observations emphasize the significance of the recent demonstration by McEwen and Schroeder that alfalfa is a host for the virus. Subclover, Trifolium subterraneum L., is described as an important natural host. The virus can be transmitted mechanically to some varieties of Phaseolus vulgaris L., but transmission is difficult and beans are considered unimportant hosts. Twelve legume genera are listed as hosts. There seem to be few distinct strains of the virus regularly present in the varieties of Pisum sativum L. grown in the Milton area.

The publication by McEwen and Schroeder (5) of their studies on the host range of pea enation mosaic virus (PEMV) provides a new starting point for understanding the ecology and epiphytology of the enation mosaic disease of peas. They have proved that alfalfa, long a suspected host, actually is a host of the virus. This paper is presented to supplement the excellent work of these authors, who have extended the known host range of the virus, and to add other plants that have been proved to be hosts during our studies of legume viruses in the Milton, Oregon and Walla Walla, Washington, pea growing areas.

SURVEY EVIDENCE THAT ALFALFA IS A HOST OF PEA ENATION MOSAIC

Approximately 100,000 acres of garden peas are planted yearly along the west slopes of the Blue Mountains between Pomeroy, Washington and Pendleton, Oregon (Fig. 1). As peas can be grown at altitudes ranging from 1000 to 3500 feet it is possible to prolong the harvest season by varying the dates of planting at the different elevations. Peas are harvested from June 1 until August 1 and are either frozen or canned.

The pea aphid, Macrosiphum pisi Kalt., has been a source of serious damage to pea crops here for many years. During the early days of the industry the damage was attributed to the feeding of the pea aphids. While it is true that this insect has occasionally increased so rapidly that the peas are dried up by aphids sucking juices from the plants, that situation seldom occurs. Usually the aphid populations are much lower, and the damage is caused by the viruses which they carry. In 1954, although control of the pea aphid with parathion was so complete that it was possible to visit field after field where only small numbers of aphids remained, 25 percent or more of the crop was lost from viruses, chiefly pea enation mosaic virus (PEMV).

Approximately 10,000 acres of irrigated alfalfa grown for hay or seed lie about 12 miles from the center of the pea area (Fig. 1). The pea aphid, which is the chief vector of the pea viruses in this area, lives on this irrigated alfalfa throughout the year. During spring, aphids may become so abundant on alfalfa that the excess population moves into the pea fields in May. The winged aphids stop for a short time on a pea plant, deposit a few young, then fly on to other plants. Thus a single winged aphid may distribute its progeny, as well as any viruses it may carry, through several fields. Wind and rain storms, common in spring, frequently knock the progeny from the original plant; later the insects may crawl back on other plants. This very often results in the inoculation of a group of adjacent plants by the progeny of one winged aphid. As the peas mature, another generation of winged aphids appears, leaves these plants,

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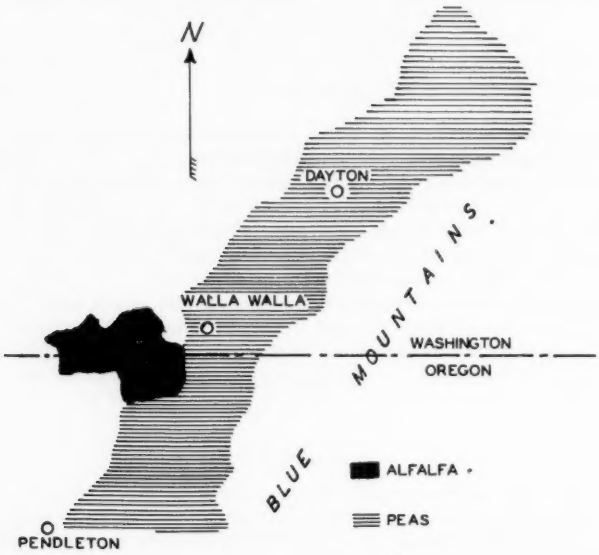


FIGURE 1. Diagrammatic map showing position of 10,000 acres of alfalfa relative to 100,000 acres of garden peas.

and moves to later peas. This movement from early to later peas results in the transfer of a much larger quantity of virus than that originally brought in, presumably from alfalfa. For this reason, virus damage is most severe on late peas.

In addition to the irrigated alfalfa, small patches of dryland alfalfa may be grown close to the pea fields, for erosion control or for one cutting of hay in June. These patches become so dry by midsummer that the pea aphid rarely overwinters in them and very few or no aphids are present in the early spring. They are reinfested each year by migrant aphids at the same time as the nearby pea fields are infested. However, because they are so close to the pea fields, they may be temporarily infested with aphids from the maturing fields and thus be inoculated with PEMV from the local peas. These alfalfa patches may then assume importance as reservoirs of pea enation virus in strategic locations.

Volunteer garden peas very rarely survive the winter in the Blue Mountain area, but field peas, Pisum arvense L., when planted in the fall, normally do live over winter. These occasionally have been found infected with PEMV. Field peas are usually planted too late in the fall to receive a fall infestation of aphids, but may receive both aphids and virus early in the spring when some winged aphids are produced on alfalfa. Aphids produced on field peas may carry enation mosaic into garden pea fields to leeward. The exact role of field peas in the aphid-virus complex is not well understood, but the distribution pattern of the comparatively few fields precludes their being the principal source of the virus.

The use of modern herbicides and excellent cultural practices have kept the roadsides and fencerows remarkably free from weeds including Trifolium spp. Only a few escaped plants of Melilotus spp. or alfalfa are found on the roadsides, too few to be important sources of inoculum.

Field studies have proved that the irrigated alfalfa is certainly the principal source of the aphids that initially infest the peas. Following the 1954 experience, when good control of aphids in pea fields failed to prevent serious losses from virus, pea growers and processors organized a community program to control aphids on irrigated alfalfa in early spring before they reach the peas. Losses from PEMV have been much less since that program was started.

These observations indicate that alfalfa usually is the initial source of PEMV in the Blue Mountain area. It is the only plant in the area that meets the dual requirements of being abundant and of being a natural host for both aphids and virus (5). Further confirmation is the geographic distribution of early virus infections in peas. The prevailing wind movement in

spring is from the west-southwest. Early virus infections are most prevalent to the leeward of the irrigated alfalfa. Infections become less common as the distance from alfalfa becomes greater or as the incoming aphid population from alfalfa becomes smaller. In a large area northeast of Pendleton, Oregon, out of line with the direct movement of aphids from the chief alfalfa areas, only a small number of aphids are present and little or no virus infection develops in most seasons. Virus infection is severe in this area only when large numbers of aphids move into it from other pea growing areas in which early infections were prevalent.

Every year, while searching for the source of PEMV, samples of alfalfa plants selected from various alfalfa fields were used for mechanical inoculations, always to broadbean, Vicia faba L., and sometimes to peas also. During the winter in some years overwintering aphids were collected from alfalfa plants and transferred to broadbean test plants. We have never found an alfalfa plant that showed symptoms acceptable for PEMV, nor have we recovered the virus from any alfalfa plant nor from any aphid that was overwintering on alfalfa. We have, however, many times recovered both alfalfa mosaic virus and PEMV from pea plants obviously infected with PEMV: circumstantial evidence that both viruses had been transmitted from alfalfa. Therefore we welcome the proof by McEwen and Schroeder that alfalfa is actually a host of PEMV. Their demonstration that the virus is usually symptomless in alfalfa explains, also, why a search for infected plants among 10,000 acres of alfalfa is likely to fail, as it did for us.

CRITICAL SYMPTOMS OF PEA ENATION MOSAIC VIRUS INFECTION

Further discussion of the host range and strains of PEMV requires consideration of those symptoms that conclusively demonstrate the presence of the virus. The definitive symptoms of infection by a plant virus, developed in a suitable host species in proper state of growth, culture, and environment, are consistently expressed as varied patterns of chlorosis, as necrosis, and as alterations in the form of the plant or its organs. The changes in form result from highly specific hypoplasias and hyperplasias induced by the virus. Chlorosis and necrosis are usual responses to legume viruses, but changes in plant form are rare. Fortunately, specific hyperplasias (6, 7) characterize PEMV in several of its hosts which are therefore usable as critical test plants. All strains of PEMV that we have isolated can be identified as PEMV by inoculating them to Perfection peas or to small-seeded broadbeans. On these test plants they produce characteristic unique hyperplasias that appear superficially as enations, epidermal overgrowths, or fleck-like windows. The term "enation" is usually misleading with respect to symptoms in peas and broadbean, because true enations or "little leaves" are seldom produced. The word "enation" connotes tiny leaf-like structures composed of chlorenchymatous tissues. Typical enations do form regularly on "Telephone peas" as formerly grown on the Oregon coast, and on crimson clover, Trifolium incarnatum L. On the test plants, however, and on Thomas Laxton, Alaska, and most dwarf peas, the so called enations are merely overgrowths of the epidermal cells into rough ridges along the underside of veins (Fig. 6). Pea enation mosaic virus seems to be the only legume virus that produces this hyperplasia of the epidermis of the veins.

The most specific symptom of PEMV is the development of conspicuous hyaline window-like areas in the leaves, produced by sheets of highly specialized cells that extend from veins and veinlets and have the form of a true neoplasm (7). These cells are without chlorophyll and are compacted into flat masses that replace the chlorenchymatous tissues and appear as translucent windows; the epidermis is also involved (Fig. 5). This is the specific symptom of this virus in several of its hosts (Fig. 2), and is universally recognized as a significant symptom of PEMV (4). The hyaline areas are comparatively large in susceptible species of Vicia, Pisum, and Lathyrus; they are small but regularly present in susceptible species of Trifolium. This manifestation is nicely illustrated by Quantz (12, Fig. 1, page 25).

The disturbances of chlorophyll formation connoted by the terms "mottle" and "vein-clearing" occur regularly during the early stages of the disease in most of its suscepts (see summary of symptom progression by Koehler and Klinkowski (4)) but possess little specific diagnostic significance for PEMV. Necrosis, on the other hand, is unusual for this virus except in crimson clover. Within a population of infected crimson clover plants necrosis occurs in the foliage of many individuals. We have found that crimson clover is a poor plant in which to maintain the virus because many of the inoculated plants are severely weakened or even killed by the necrotic effects of the virus in that host.

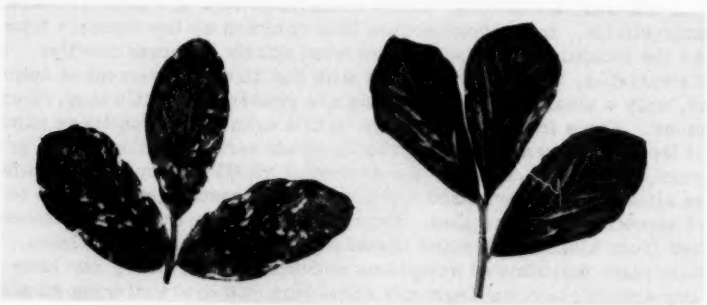


FIGURE 2. Broadbean leaves. Comparing classical window-like symptoms of enation mosaic virus (left) with chlorotic patches characteristic of silky green virus (right). The latter may be a strain of the enation mosaic virus.

FIGURE 3. Extreme interveinal chlorosis produced by enation mosaic virus in variety OSC 9 peas.

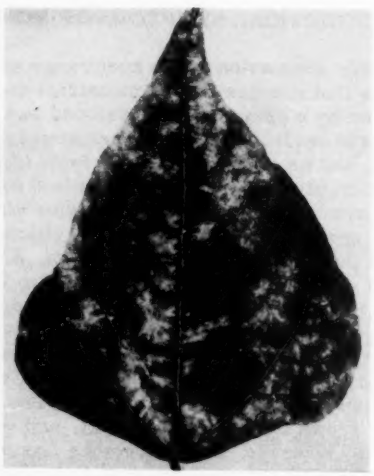


FIGURE 4. Bountiful bean infected with enation mosaic virus by mechanical transfer from peas.

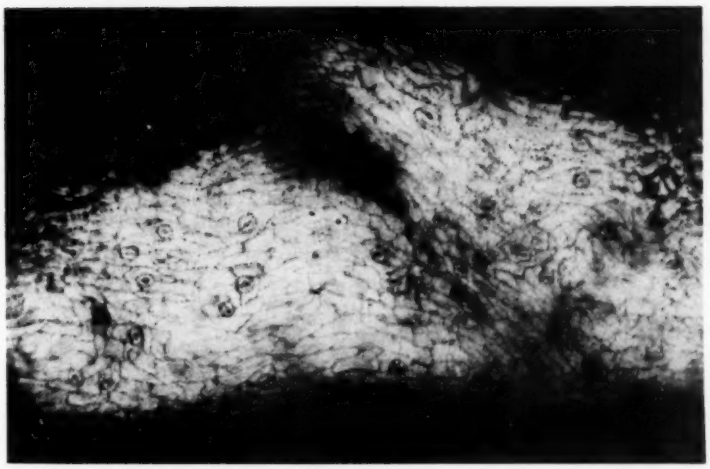


FIGURE 5. Window-like structure formed by hyperplasia of cells in leaf of Perfection pea infected with enation mosaic virus. About 97.5X.

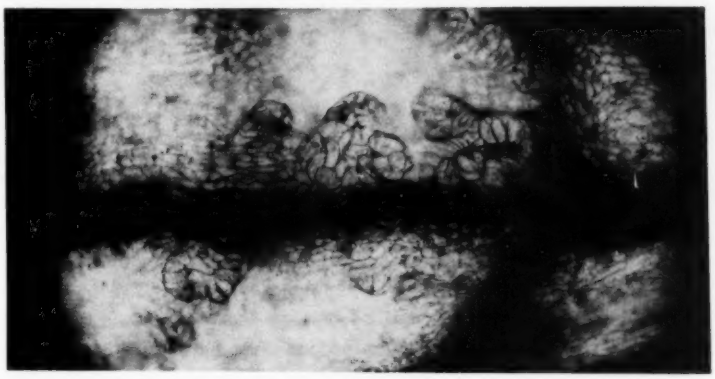
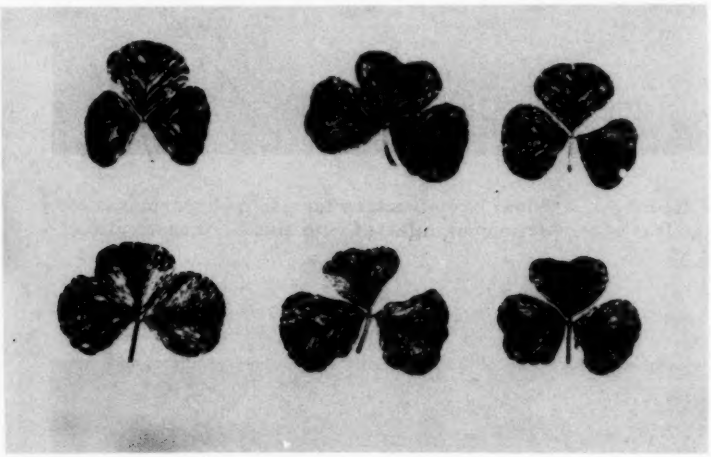


FIGURE 6. Epidermal enations formed on vein on lower surface of Perfection pea leaf. About 75X. Note also hyaline windows extending out from edge of vein.

SUBCLOVER, A HOST OF PEMV[4]

We learned that subclover, Trifolium subterraneum, is a host for PEMV when Dr. John R. Hardison discovered naturally infected plants in his variety trials at Corvallis in 1949. The symptoms are almost identical with those in crimson clover except that necrosis develops in comparatively few plants. Sometimes true enations are formed and these are relatively large. The principal symptom, however, is the development of the hyaline hyperplasic areas characteristic of the virus (Fig. 7). Mechanical inoculations to subclover from peas naturally infected with PEMV are as uncertain in this host as in other plants suitable for isolation and detection of the virus. Table 1 records inoculations made during our 1951 survey in which Mt. Barker subclover was used along with other test plants to isolate PEMV from naturally infected peas.



SUB CLOVER ENATION MOSAIC FROM J. R. HARDISON'S PLOT MAY 1949-47

FIGURE 7. Typical symptoms in subclover following natural infection by pea enation mosaic virus.

Table 1. Transfers of pea enation mosaic virus from naturally infected peas.

Source: Infected pea No.	Successful transmissions over number of plants inoculated: Sub-clover	Broad bean	Alah peas	Perfection peas
899	1/14	2/7	-	-
900	0/9	0/4	0/19	-
901	2/12	0/4	0/16	-
903	0/6	0/6	0/6	-
904	0/8	1/8	-	0/8
907	0/9	0/8	-	1/6[a]

[a]Inoculations from this PEMV infected pea test plant to subclover gave 2/6 transfer.

[4]Since this paper was written we have seen Dr. Hagedorn's Abstract (Phytopathology 47: 14, 1957) reporting new hosts of PEMV determined by inoculation. Subclover is cited in his list.

Table 2. Proved hosts of pea enation mosaic virus.

Technical name[a]	Common name	Infection recorded	
		From inoculation only[b]	Natural infection[c]
Astragalus rubyi Gr.-Morr.*	Milk vetch	X	
Cicer arietinum Linn.*	Chick-pea	X	
Glycine max (L.) Merr.	Soy bean	X	
Lathyrus cicera Linn.*	Flat pod Pea vine	X	
L. hirsutus Linn.	Rough pea	X	
L. latifolius Linn.	Perennial pea		United States
L. odoratus Linn.	Sweetpea		United States, Europe
L. tingitanus Linn.	Tangier pea		Europe
Lens culinaris Medic.	Lentil		Europe
Lupinus albus Linn.*	White lupine	X	
L. angustifolius Linn.*	Blue lupine	X	
L. luteus Linn.	Yellow lupine		Europe
Medicago arabica All.	Spotted bur clover		United States
M. hispida Gaertn.	Bur clover	X	
M. sativa Linn.	Alfalfa		United States
Melilotus alba Desr.	White sweet clover	X	
M. officinalis (L.) Lam.	Yellow sweet clover		United States
Phaseolus vulgaris Linn.	Bean	X	
Pisum sativum Linn.	Pea		United States, Europe
P. sativum var. arvense (L.) Poir.	Field pea		United States, Europe
Trifolium glomeratum Linn.*	Cluster clover	X	
T. incarnatum Linn.	Crimson clover		United States, Europe
T. repens Linn.	Ladino clover	X	
T. repens	White clover	X	
T. resupinatum Linn.*	Persian clover	X	
T. subterraneum Linn.	Subclover		United States
Vicia faba Linn.	Broadbean		Europe
V. narbonensis Linn.	Narbonne vetch		Europe
V. pannonica Lapevr.*	Hungarian vetch	X	
V. sativa Linn.	Common vetch		United States, Europe
V. villosa Roth.	Hairy vetch		United States, Europe

[a]The binomials were kindly checked and edited by Dr. Albert N. Steward. The asterisks indicate species added from Hagedorn's abstract (Phytopathology 47:14, 1957).

[b]The symbol X indicates that the host has been proved only by inoculation. All cited species are certainly hosts, with the possible exception of soybean, which, however, has been listed by four American investigators as a host. The careful work of Quantz has failed to confirm its susceptibility and we have not tested it because all seedlings, before they were inoculated, showed symptoms like those previously reported as proof of the presence of the virus.

[c]Regions where natural infection has been observed or recorded. The European hosts listed are those reported by Quantz (12).

Subclover is an excellent host in which to maintain the virus for experimental studies. The seedlings should be in at least the four-leaf stage before attempting to inoculate them. A gentle inoculation with the spatula technique (8) is advisable. Initial veinclearing appears in about 13 days and is followed by formation of characteristic hyaline areas. Plants grow to about one-third normal size, developing progressively stronger symptoms as they age, but will remain alive and usable as source plants for 6 to 8 months. The symptoms produced by the bean yellow mosaic, alfalfa mosaic, and western ringspot viruses are distinct in this host and cannot be confused with PEMV.

PHASEOLUS VULGARIS A POSSIBLE HOST

The record by Johnson and Jones (3) of successful inoculation of Corbett Refugee beans with PEMV is very uncertain and has not been accepted by European investigators. The symptom recorded is "mild mottle" and there is no record of the recovery of the virus from the beans. Mild mottle is definitely not a conclusive symptom of PEMV and beans have long been considered unproved as hosts of this virus.

Our first successful transfer of PEMV to Phaseolus vulgaris L. was accidental. During the 1953 survey we were particularly interested in isolating other viruses present along with PEMV in pea plants that showed wilt and necrosis as well as definite PEMV symptoms. For this purpose we used Bountiful beans in addition to the usual test plants. In one series, a collection of the Perfected Freezer variety of pea showing both typical PEMV symptoms and certain necroses gave four successful transmissions in four inoculations to bean, evidenced by the yellow net vein symptoms shown in Figure 4. We recovered PEMV from one of these Bountiful bean plants by subtransferring to broadbean. The yellow net vein symptoms appeared in Bountiful beans inoculated from two other naturally infected pea plants during our 1953 studies, but PEMV was recovered only from the series mentioned above. That the net vein symptoms induced in the beans are not due to viruses other than PEMV present in the pea source plants is indirectly indicated by the fact we were unable to transfer from Bountiful bean to Bountiful bean. If the net vein symptoms were due to strains of alfalfa mosaic virus or western ringspot virus that are commonly present in peas infected with PEMV, the bean to bean transfer should have been close to 100 percent. Inoculations made since 1953 from naturally infected peas have twice induced the yellow net vein symptoms in Bountiful beans and once in Topcrop, but PEMV has been recovered and proved only once, as mentioned above. These data indicate that Phaseolus vulgaris is an unimportant host of the virus.

HOST RANGE DISCREPANCIES

As pointed out by Quantz (11), inconsistencies in the properties of the virus as reported by different investigators are more likely attributable to difficulties in working with the virus than to true differences in the virus entities tested; that is to say, that inoculation failures rather than inherent variations among divergent strains of the virus account for such discrepancies as the differences of 18° C for the thermal inactivation point reported by different authors! The same situation applies even more emphatically to the host range as determined by different investigators. For example, Simons (13), using insect vectors and working in California, lists the broadbean, a generally recognized host, as insusceptible to the virus. McEwen and Schroeder state they have not observed field peas naturally infected in New York, yet field peas are important natural hosts of the virus in the Pacific Northwest and in Europe. Some workers have relied on aphids to transfer the virus; others have used carborundum techniques. The age of the test plants, the methods used, and the care taken in making inoculations are critical factors that affect transfer. Positive results are obviously more significant than negative results in determining the host range for this virus. The hosts that we consider as proved are listed in Table 2.

STRAINS OF PEA ENATION MOSAIC VIRUS

We have occasionally found some strains of PEMV that exhibited unusual symptoms in some hosts, but these strains met our specific symptom criteria when inoculated to the test plants. However, we have not observed such an array of divergent strains as characterizes the bean yellow mosaic virus. The latter virus has a far larger host range which includes, besides legumes, several species in the Iris family. The only other virus we have studied that produces symptoms similar to those of PEMV is the narcissus mosaic virus. The hyper-

plasia that results in the roughening of narcissus leaves is structurally very similar to that produced by PEMV in its legume hosts. The narcissus virus is aphid-borne but probably not aphid-perpetuated. Attempts to transfer narcissus mosaic virus to broadbean have failed[5]. This point is made to emphasize the fact that PEMV is a specialized virus confined to comparatively few hosts. Because of what may be called the plant-effect that gradually changes a virus, one may hazard the opinion that the larger the host range of a virus the greater the number of strains that will become apparent.

Osborn (10) first showed that the symptoms produced by a continuously aphid-transmitted isolate (strain) of PEMV differed markedly from those produced by transferring the virus mechanically. That is to say that, for the same virus, symptoms resulting from mechanical transmission were different from those resulting from aphid transmission. We compared the effects of a typical isolate on peas by aphid vs. mechanical transmission. The aphid transmission produced much more severe symptoms than the corresponding mechanical transmission. Such differences could be mistaken for effects due to different strains of virus.

Another cause of misinterpretation when looking for divergent strains of PEMV is the fact that this virus exhibits what may be called a "carrier virus property" (9). Thus, when an aphid takes up this virus from a host plant containing other viruses that are normally not aphid-perpetuated or readily aphid-borne, the presence of PEMV seems to exert some stabilizing effect that increases the ability of the aphids to transmit the second virus. Such a situation was first demonstrated by Smith and Lea. (14). During our survey of peas we have found a medley of symptoms in PEMV-infected plants that later analysis proved were partly due to the presence of other viruses. Where top wilts and coarse mottles are present along with specific symptoms of PEMV one should test for a second virus even though aphid transfer from pea to pea produces the same symptom complex.

Interpretation of occurrence in peas of necrotic or killing strains apparently of this virus is especially difficult. Necrosis, as mentioned previously, is severe in some individuals among a seedling population of crimson clover plants affected with the virus. This variation in occurrence of necrosis is explainable as a function of genetic variation in the host. In leaves of broadbean plants that have been infected with the virus for several months necrosis often occurs on the edges of the old hyperplasic "window" areas. Osborn (10), who used two collections of the virus maintained at Princeton, New Jersey, for several years, decided that he had two strains because one produced necrosis in broadbean and the other did not. Since both were alike in peas he considered the difference minor. There is certainly a considerable variation in the tendency of different collections to produce necrosis in broadbean. When necrosis is conspicuous in the terminals of pea plants that show PEMV symptoms also, we have usually isolated alfalfa mosaic virus, western ringspot virus, or some undetermined virus. In our opinion the question as to whether some strains of PEMV are necrotic in peas needs further investigation, especially with regard to the reactions of different pea varieties to the same strain of virus.

Every year during the past six years, we have isolated from peas that certainly were affected by enation mosaic a virus to which no specific name has been assigned. It has been detected only in broadbean in which it produces silky green streaks (9). Frequently where five 2-inch high broadbean seedlings were inoculated from naturally infected peas, one out of the five developed typical PEMV symptoms, and three the silky green; sometimes both viruses were apparent in the same test plant. The silky green areas are characterized by slight cell rearrangements that indicate relationships with PEMV. The symptom usually requires 30 to 40 days for expression. We have been able to obtain 100 percent transmission of this virus from broadbean to broadbean, but never to any other plant including peas. Because of the long incubation period, satisfactory protection tests between this unknown virus and PEMV have not been made, since neither virus can be inoculated easily to old plants. The most probable hypothesis is that the silky green virus combines with PEMV in natural field infections to produce symptoms that could be interpreted as due to different strains of the latter virus. We have not had proper facilities to test this hypothesis.

During the latter years of the survey one general change in symptoms has occurred that does suggest the development of a distinct strain of the virus. A large percentage of the infected plants now exhibit comparatively few "windows" but are conspicuous because of yellowing and thickening of the veins of the leaves and some blanching of the areas between the veins

[5]The juice of narcissus foliage is extremely toxic to broad bean leaves and all attempts to relate the viruses have been affected by the incompatibility of test plants. We do not consider the failure of such inoculations to be proof that the viruses are unrelated.

(Fig. 3). Profound hyperplasic changes deform the pods in a manner characteristic of the former type strains. These new symptoms are most noticeable in the new lines of Perfection Freezer. Since typical symptoms are obtained following mechanical transfer to broadbean, the new symptoms, however, may be associated with the new varieties of peas rather than with distinct strains of the virus.

Further evidence that strains of the virus are not, as yet, widely divergent and accompanied by profound physiological differences, is given by results of tests with pea varieties resistant to PEMV. Several lines of resistant peas developed by Dr. W. T. Schroeder at Geneva, New York have been tested by Dr. W. A. Frazier at Corvallis, Oregon and by the authors at the United States Department of Agriculture Entomology Laboratory at Walla Walla, Washington. In both locations the resistant peas were exposed to highly viruliferous aphids under conditions where the control plants were nearly 100 percent infected. None of the Geneva lines developed the disease. Thus the differences between strains of the virus in the Washington and Oregon locations were not such that they could break through the resistance barrier developed against the strains of the virus present in New York. Much more work should be done on the strain problem in PEMV, but the present evidence is that the virus is remarkably constant wherever it occurs.

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OREGON AGRICULTURAL EXPERIMENT STATION; AND UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE

TOMATO ASPERMY VIRUS IN CHRYSANTHEMUMS FROM ASIA AND EUROPE

Philip Brierley[1]

In September 1956 and in April and August 1957 John L. Creech sent us 153 chrysanthemum varieties recently imported from Japan and held under quarantine at the Plant Introduction Station, Glenn Dale, Maryland, with the request that we index them for important virus diseases before their release to American growers. One or more plants of each variety were indexed by juice inoculation to tobacco to detect tomato aspermy virus (1, 2) and by graft inoculation to the chrysanthemum test varieties Blazing Gold and Golden Mistletoe to detect the viruses of chrysanthemum stunt (2), flower distortion (1, 2, 3), aster yellows (2), or chrysanthemum mosaics (1, 2) and rosettes (2). For comparison we indexed, for aspermy virus only, 15 varieties from Taiwan and 30 varieties from England, recently imported by H. M. Cathey, and not intended for introduction to American growers.

Twenty-four of the 153 Japanese varieties (16 percent) yielded tomato aspermy virus, 22 (14 percent) mosaic viruses, and five (3 percent) chrysanthemum stunt virus. None of the Japanese chrysanthemums yielded other viruses previously known in American stocks. The low content of stunt virus seems to indicate that this North American virus was introduced into Japan with some of the American varieties used by Japanese cut-flower producers. The mosaic viruses from Japan, like those previously encountered, differ widely in virulence to the Golden Mistletoe test variety. Except for the relatively high content of aspermy virus, this sample of Japanese chrysanthemums is more nearly free of important virus diseases than garden varieties from our own country or from Europe that we have sampled.

The Japanese varieties found infected with aspermy virus were Ashina-No-Yuki, Benitaiyo, Dai-Higyo, Eieno-Izumi, Hakucho, Haku-Sei, Hime Komachi, Hosizukiyo, Kenkokune-Sidameri, Kingekko, Kizashi, Konagare, Kotiyounomai, Okinanotomo, Renkano-Toma, Seikonouma, Shuokan, Sinrokuna-Tuya, Takino-Monizi, Tengokuno-Shirabe, Ten-Kei, Tomari, Torinyumon, and Tshibumi.

Of the 15 varieties from Taiwan tested five (33 percent) were infected with aspermy virus as follows: Autumn Hat, Buddhist Priests' Gown, King in White, Roc's Cry, and Spring under the Pine. Of 30 tested English varieties four (13 percent) were infected with aspermy virus as follows: Chatworth, Royal Pearl, Salmon Sweetheart, and Sweetheart.

Ten Japanese varieties of which two or more plants were indexed proved only partially infected with aspermy virus, and three varieties were found partially infected with stunt virus. This indicates that the aphid-borne (4) aspermy virus and the contact-transmitted (2) stunt virus are spreading in Japan. Since tomato aspermy virus is slow to become systemic in chrysanthemum (1), recent infections are not surely detected by indexing. Duplicate index tests made when the chrysanthemums were in vigorous vegetative growth agreed well in general. However, the first index test of ten garden varieties in September 1956, when the chrysanthemums were potbound, failed to detect the virus. Repeat tests made in April 1957, after the plants were repotted and fertilized, revealed aspermy virus in three of ten varieties. One plant each of the varieties Hakucho and Kingekko and two of Shuokan yielded no virus in the April tests. These supposedly healthy plants were propagated and their propagations were reindexed in June. All such propagations of Hakucho and Shuokan proved virus-free in two further trials, but all plants of Kingekko proved to carry a mild strain of aspermy virus that expressed symptoms in tobacco after 9 to 15 days instead of 6 to 10 days, the usual period for more virulent strains.

It seems likely that tomato aspermy virus is of east Asiatic origin because (a) the cultivated chrysanthemum is of such origin and greater quantities of this plant have moved out of Asia than have returned there; (b) the virus is prevalent in Japanese and Taiwan chrysanthemums and differentiated into strains in these plants; and (c) most varieties from east Asia are not visibly injured by aspermy infection, suggesting long association, selection or adaptation to this virus. Of 20 Japanese varieties, five Taiwan varieties, and two English varieties that were aspermy-infected and permitted to flower, none showed recognizable distortion of the blooms except three Japanese varieties of the anemone class (Kizashi, Tomari, and Tshibumi), which had some irregularity and twisting of their ray florets.

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It is unfortunate that aspermy virus continues to enter the United States in chrysanthemums from various other countries. This aphid-borne virus is severely damaging to tomato, one of the most popular and most rewarding home garden vegetables. Since the home gardener commonly grows chrysanthemums and tomatoes within the same limited area, and since he rarely achieves effective control of aphids, aspermy adds a new disease hazard to those previously confronting him. It should be remembered that only in United States do we have commercial propagation specialists (2) equipped to screen chrysanthemums for disease content. Importers would be wise to have chrysanthemum introductions screened by one of these firms before releasing these plants to the American public. A collection of most of the better Japanese chrysanthemum varieties, held by the New Crops Division, United States Department of Agriculture, will be indexed and those found to be virus-free released to growers.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

ACCENTUATION OF BLUE ALBEDO IN MARSH GRAPEFRUIT BY SIZING SPRAYS WITH 2,4,5-T

J. B. Carpenter[1] and H. Z. Hield[2]

Stubborn disease of citrus is probably caused by a graft-transmissible virus[3]. The best diagnostic symptom of stubborn is acorn-shaped fruit, but this symptom is expressed erratically. Thus, other symptoms must often be used in the tentative identification of this disease. Among these is a blue stain in the albedo of citrus fruits, especially grapefruit, that is believed to be a characteristic symptom, or perhaps even a specific indicator, of stubborn. Blue albedo has been associated with the stubborn disease in grapefruit for many years[4], and since 1954 it has been found in individual trees of 26 varieties of grapefruit in Arizona, California, Florida, and Texas, as well as in some orange and tangelo varieties (Carpenter, unpublished data). Up to now, blue albedo has not been associated with any other disease or physiological disorder of citrus.

Small fruits with blue albedo were unusually abundant at harvest in old-line Marsh grapefruit trees sprayed with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) in experiments conducted by Hield to study the effect of plant-growth-regulator sprays on fruit size. Most old-line Marsh trees are believed to carry stubborn disease and the frequent presence of fruit with acorn shape or blue albedo on the trees of this experiment indicated that stubborn was generally present. The following data were obtained to help determine the true relation of blue albedo to stubborn disease.

Table 1. Incidence of abnormal fruits with symptoms of stubborn disease on Marsh grapefruit trees treated with growth-regulator sizing sprays, Riverside, California, 1955-1956.

Treatment	Number single-tree replicates examined	Number fruits examined per tree	Abnormal fruits with symptoms of stubborn			
			Diameter less than 2 inches	Diameter 2 inches or more	Total	
			Number	Number	Number	Percent
Control, no spray	5	516	10	10	20	4
MCPA, 24 p.p.m. (low volatile ester)	2	676	17	14	31	5
2,4-D, 24 p.p.m.:						
Acid emulsion	2	535	20	9	29	5
Low volatile ester	2	531	15	12	27	5
2,4,5-T, 12 p.p.m.:						
Acid emulsion	5	543	69	10	79	15
Low volatile ester	5	515	134	18	152	30

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[2]Associate Specialist, University of California Citrus Experiment Station.
[3]Fawcett, H. S. 1946. Stubborn disease of citrus, a virosis. Phytopathology 36: 675-677.
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A comparison of the effects of three growth regulators in six treatments (Table 1) on the sizing and growth characters of Marsh grapefruit was begun in June 1955. The materials were applied as single drenching sprays to 22-year-old Marsh grapefruit trees in one row, comprised of seven replicates each containing the six treatments as randomized single-tree plots. Harvest data were obtained in May 1956, but only from replicates 1 through 5 because of an error in picking the others. All fruits from certain individual trees were examined. The abnormal fruits, resembling those from grapefruit trees believed to have stubborn disease, were separated into two classes: those 2 inches or larger in diameter and those less than 2 inches. The abnormal fruits included all those with acorn-shape or blue albedo and all that were small, hard, and usually rough. When cut, these latter fruits displayed a dense albedo in which the vascular system was prominent and often accompanied by a pronounced bluish stain.

The results of the several treatments are given in Table 1. Applications of 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl, 4-chlorophenoxyacetic acid) did not increase the number of fruits showing symptoms of stubborn. The 2,4,5-T treatments increased greatly the numbers of stubborn-like fruit; 87 percent of the affected fruits were mostly much smaller than 2 inches in diameter. This retention of smaller fruits may be significant, for the bulk of these were dense and rough and many had blue albedo. They resembled fruits that might have been shed during the "June drop" if abscission had not been arrested. However, the total numbers of fruits on these trees and on the controls were about equal.

These results raise some questions about the relation of blue albedo to stubborn disease. Does 2,4,5-T accentuate an effect induced by the virus causing stubborn disease, or is blue albedo a physiological condition induced by a plant growth regulator and aggravated by stubborn disease? Is blue albedo a specific indicator of stubborn disease or only a characteristic but non-specific symptom? The common occurrence of blue albedo in grapefruit varieties that are now available would probably vitiate experiments designed to answer these questions. However, with the onset of fruiting in nucellar grapefruit clones or seedlings of recent origin, believed to be free of stubborn, suitable experiments with growth regulators should be undertaken and observations should be made on the incidence of blue albedo in the fruits of untreated and uninoculated plants. Studies of seed and insect transmission may be required before this part of the stubborn-disease problem can be clarified.

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DECLINE OF STANLEY PLUM ACCENTUATED BY WINTER INJURY

J. D. Kirkpatrick[1], K. G. Parker[2], and E. G. Fisher[1]

Abstract

A new and distinctive leaf pattern associated with decline and collapse of Stanley plum trees on Myrobalan rootstock (Prunus cerasifera seedlings) is described. Trees having this leaf symptom also had a constriction, an inverted shoulder, at the bud-union which developed as a result of what appeared to be differential winter injury to tissues above and below the bud-union. Both the leaf symptom and the constriction of the bud-union appear to be an expression of some factor which causes an increased susceptibility to winter injury.

In recent years the decline of Stanley plum trees propagated on Myrobalan rootstock (Prunus cerasifera) has become a problem in New York orchards (1, 2). The affected trees in the orchard examined in the present study are in poor vigor with their leaves cupped, rolled upward, and showing a distinctive chlorotic pattern. Some affected trees eventually die. The affected trees are scattered throughout the orchard and no clear correlation exists relating tree decline and collapse to soil structure, effective soil depth, soil pH, or to the readily available soil nutrient level.

HISTORY

In 1950 Brase (1) suggested that the constriction disease of Stanley plum, which had become apparent since 1943 in New York orchards, might be due to an introduction of a virus or virus-like factor into the propagating material. He suggested that the virus-like factor may have suddenly appeared in the rootstock because of a change in the source of seed, or because an injurious factor may have been introduced and carried in the bud-wood. The possibility of a virus-like factor in the rootstock appears similar to the problem Proebsting and Hansen (3) found in orchards of 25-year-old apricot trees propagated on Myrobalan rootstock in California. Their observations on the decline of apricot trees bear a strong similarity to the symptoms observed in the Stanley plum decline. They found that the incidence of apricot decline was negatively correlated with scion rooting. Correction of affected apricot trees was obtained by inarching with apricot seedlings. It was suggested that since the incidence of apricot decline could not be associated with any orchard condition but was limited to trees in a small district which were planted about the same time, the causal factor may have been introduced from an unusual source of Myrobalan seed. Brase and Parker (2) suggested that the decline of Stanley plum is quite similar to the problem with clone 227 of Northern Spy apple (4). When this clone was used as a rootstock for two different McIntosh clones, trees of one clone died in a few years whereas trees of the other clone did not. Tukey and Brase (4) suggested that the uncongenial McIntosh clone may have carried a virus.

FIELD TRIAL

In the summer of 1957 a 5-year-old planting of 685 Stanley plum trees on Myrobalan plum rootstock had 190 trees in some stage of collapse. By September 28, 53 trees had rapidly wilted, collapsed, and died without abscission of leaves or fruit; 46 were in a late stage of collapse; 48 were in an intermediate stage; 33 were in a beginning stage; and 10 were possibly in a beginning stage. Of the 53 trees that wilted, collapsed, and died, 28 collapsed by June 20, 17 by July 16, and 8 by August 30. The designation of stages of collapse was based upon the degree of expression of a distinctive leaf pattern peculiar to this condition and upon the incidence of an inverted shoulder at the scion and rootstock union. These two conditions will be discussed below.

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Prior to collapse the leaves of affected trees develop a distinctive pattern sequence (Fig. 1):

a) Beginning stage -- The leaves have a very slight fading of the chlorophyll in the interveinal areas while the veinal areas remain green.
b) Intermediate stage -- The leaves have a distinctive yellow interveinal coloration tending toward an orange hue while the veinal areas still remain green.
c) Late stage -- The leaves have yellow-orange and brilliant red interveinal coloration while the veinal areas remain green. The veinal areas then fade and the whole surface is yellow, orange, or red. The extreme margins become uniformly necrotic.

All stages may be found on trees in b and c, but there is a predominance of one or the other pattern type depending upon the severity of collapse. It must be emphasized that this is a sequence, a dynamic change, and not distinct, separate steps.

There is associated with the leaf pattern an overgrowth, an inverted shoulder, at the union (Fig. 2). This is sometimes accompanied by a partial horizontal separation of the scion and rootstock at the windward side of the union. When this occurs some trees have in addition a vertical downward split of the rootstock beginning at some point along the horizontal separation.

On September 28, 15 trees were selected for study. Trunk segments at the union were sawed in radial and cross section, polished, and observed under a binocular microscope. The trees selected were of the following types (Figs. 3 and 4): five trees examined were classed as in an intermediate to late collapse condition, three trees examined had wilted and collapsed between July 16 and August 30, four trees examined had wilted and collapsed prior to July 16, and three trees examined were in a vigorous and apparently healthy condition.

Type 1. Trees in an intermediate to late, or late collapse condition on September 28.

Rootstock and scion produced approximately the same amount of xylem through 1955 with no serious winter injury apparent to the xylem produced during this period. Death of the scion cambium in the 1955-56 winter was evident in areas bordering the union. The callus formation about the injured area forms an enlargement of the scion above the union. Possible local invasion of fungi occurred in the callus areas. The cambium of the rootstock was still functional, but the enlargement of tissues differentiated by the rootstock cambium below the winter-killed areas of the scion was retarded. Evidence of a suture formation occurred at the junction of the scion and rootstock in the xylem produced after the 1954 growing season.

Type 2. Trees which had wilted and collapsed late, between July 16 and August 30.

Rootstock and scion produced approximately the same amount of xylem through 1955. Complete xylem killing of the rootstock was apparent in the 1955-56 winter. Xylem winter injury penetrated several inches into the 3- and 4-year-old wood of the scion. Most of the rootstock cambium was killed in the 1955-56 winter. While most of the remaining rootstock cambium was killed in the 1956-57 winter some small areas survived. There was also considerable local cambium killing just above the union on the scion in the 1955-56 winter. Possible local invasion of fungi occurred in the callus areas. The cambium has been non-functional or its activity repressed at the junction of the scion and rootstock since the 1954 season. This condition produced a suture at the union. The overgrowth or inverted shoulder at the union developed because the radial growth of the rootstock ceased after the 1955-56 winter while the scion trunk continued radial growth. The inverted shoulder appearance has been accentuated in many cases by callus tissue of the scion formed immediately above the union in cambial areas which had previously been winter-injured.

Type 3. Trees which had wilted and collapsed early, prior to July 16.

Rootstock and scion produced approximately the same amount of xylem through 1955. Nearly complete winter killing of xylem and cambium of the rootstock occurred in the 1955-56 winter. A definite, well delineated boundary was present at the union between winter injured rootstock tissues and healthy scion tissues. Little, if any, winter injury to the scion was observed. Very slight cambium activity in the rootstock was evident in 1956 and none in 1957. The union showed some evidence of suture formation in 1954-55 but the tissues were winter-killed in 1955-56. The overgrowth or inverted shoulder at

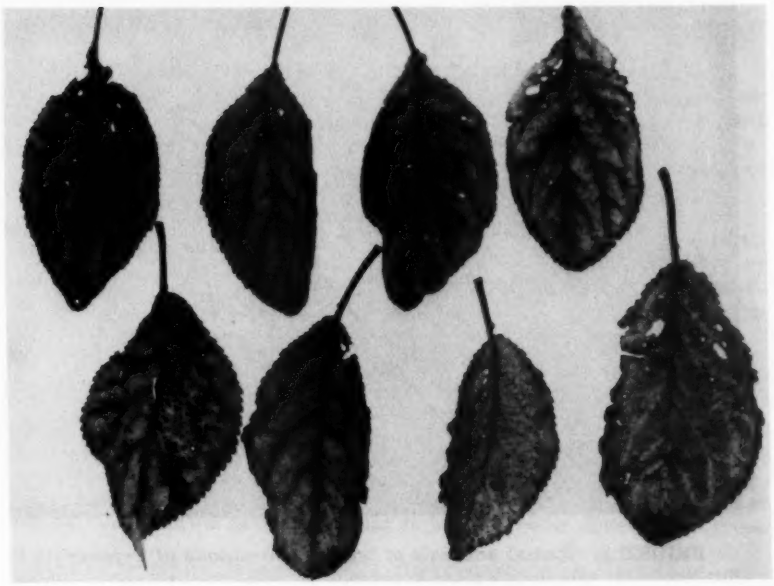

FIGURE 1. Progression of leaf symptoms associated with the decline and collapse of Stanley plum trees on Myrobalan plum rootstock. The beginning stage is illustrated at upper left through the intermediate stage at upper right to the late stage at lower right (see text).

FIGURE 2. Bud-union areas of 5-year-old Stanley plum trees on Myrobalan plum rootstock. Left, from an apparently healthy tree; right, from a collapsed tree.

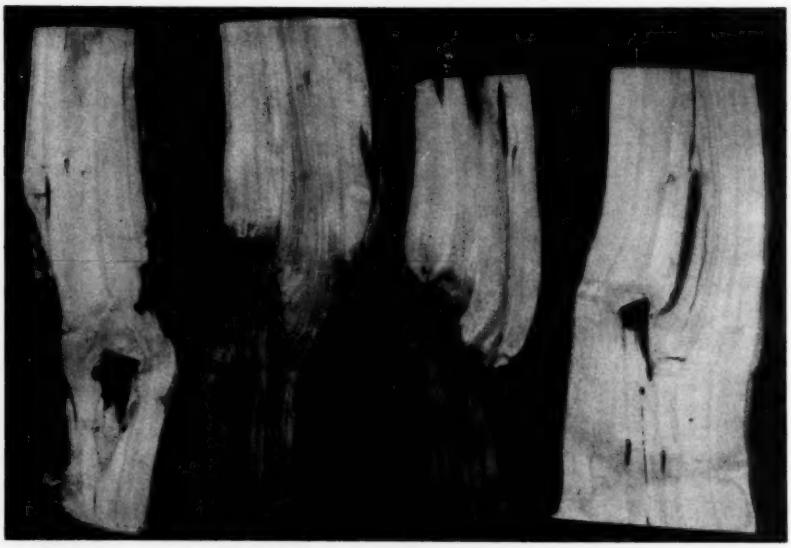

FIGURE 3. Radial sections of typical bud-unions of 5-year-old Stanley plum trees on Myrobalan plum rootstock showing decline and collapse. Left to right: tree showing severe decline symptoms on September 28, tree wilted and collapsed in late summer (July 16-August 30), tree wilted and collapsed in early summer (before July 16), and a tree in apparently healthy condition on September 28.

FIGURE 4. Bud-union areas of 5-year-old Stanley plum trees on Myrobalan plum rootstock showing collapse and decline symptoms. The bark has been removed. Trees are the opposite half of those shown in Figure 3 and are in the same order.

the union developed because the radial growth of the rootstock ceased in 1955-56 while that of the scion continued.

Type 4. Trees apparently free from collapse symptoms on September 28.

The diameter of the growth rings follows the environmental pattern. Considerably greater growth year for year was made than on trees that collapsed or had collapse symptoms. Little or no winter injury was evident after the 1952-53 winter. There was a slight but definite suture development at the bud-union. There have apparently been equal yearly increments of xylem developed in scion and rootstock.

The difference among trees in various stages of collapse appears to be directly related to the extent to which each has been winter-injured. Particularly, the extent of injury to the cambium of the rootstock and to the scion cambium just above the union in 1955-56 appears to have determined the rate of collapse and in a large measure the size and expression of the inverted shoulder at the union.

DISCUSSION

The distinctive leaf pattern associated with decline and collapse of Stanley plum trees herein described has not been found on trees winter-injured or mechanically girdled at the soil level, 3 to 6 inches above the bud-union. Girdling caused by implement damage or by "simple" winter injury to otherwise apparently healthy trees causes a general fading of the chlorophyll over the entire leaf blade. This is quite similar to the leaf color observed on trees low in nitrogen.

There appears to be suture development, a slight constriction, at the bud-union at an earlier age on the collapsed and declining trees than on the apparently healthy trees. From the magnitude of the physical expression of the suture it appears doubtful that the slight constriction could in itself induce such a great susceptibility to injury in the tissues about the bud-union. If, however, there is associated with the suture development a restriction or plugging within the conducting cells themselves, or a collapse and premature death of xylem or, particularly, phloem tissues, the effect would be root starvation and decline followed by collapse of the tree. In any event such abnormal development would induce a greater susceptibility of the affected tissues to winter injury.

The abnormal physical appearance of the bud-unions of collapsed and declining Stanley plum trees may be explained by what appears to be differential winter injury to cambium and xylem tissues. The questions as to why the tissues just above and below the bud-union were so much more susceptible to injury than other trunk regions, and why so many trees were so very much more susceptible to injury than apparently comparable adjacent trees, remain unanswered.

SUMMARY

In a field study of 685 5-year-old Stanley plum trees propagated on Myrobalan rootstock (Prunus cerasifera) 190 wilted and collapsed or showed stages of collapse during the 1957 growing season. Associated with collapse was a distinctive leaf symptom and a marked overgrowth, an inverted shoulder, at the bud-union. Stanley plum tree collapse may be explained by what appears to be differential winter injury to cambium and xylem tissues about the bud-union area. From studies of the nature and incidence of collapse it appears that some factor may induce or aggravate susceptibility to winter injury in the tissues in the area of the bud-union.

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NEW OR UNUSUAL SMALL FRUIT DISEASES AND DISEASE-LIKE OCCURRENCES IN MICHIGAN[1]

Robert H. Fulton

Abstract

In surveying for new or unusual small fruit diseases Armillaria mellea was found on the cultivated blueberry, Vaccinium australe; Monilinia vaccinii-corymbosi on pin cherry, Prunus pennsylvanica, -- first reports on these hosts. Shoestring virus was reported for the first time in Michigan on blueberry; and a new symptom for shoestring, abnormal fruit coloring, was disclosed for the Jersey variety. Fasciation was shown to be non-persistent in both black raspberry and strawberry. Aster yellows on strawberries was reported for the first time in Michigan. The occurrence of two non-parasitic myxomycetes on strawberry petioles and leaflets is presented. Finally, the infection of the originally resistant Fairland strawberry variety by Phytophthora fragariae is reported from Michigan for the first time.

For the past several years systematic disease surveys have been made in the major small fruit areas of Michigan to determine the extent of previously unreported diseases.

BLUEBERRY

ARMILLARIA ROOT ROT: In several areas of a 3-year-old highbush blueberry planting near Grand Haven, bushes in poor vigor and some apparently dead were noted. The affected bushes were interspersed in a somewhat circular shape. Wet feet, winter injury, and soil alkalinity were ruled out as causal factors. However, the grower stated that the blueberries had been planted on a clearing of second growth forest composed of oak, beech, and birch, which was suggestive of Armillaria root rot.

Rhizomorphs on the surfaces of several of the larger roots and white mycelial mats in the cortex were typical of Armillaria.

To further corroborate this finding inoculations were made in the root systems of young, potted Jersey blueberries using this fungal material. Several months later black, shiny rhizomorphs were found scattered over the root surfaces and white rhizomorphs in the cortical root areas. In addition, the plants, though adequately watered, seemed to be suffering from drought.

This was considered sufficient evidence that the fungus responsible for the symptoms was Armillaria mellea (Vahl) Quél. As blueberry is not indicated as a host of Armillaria (4) this is believed to be the first record for Armillaria on the highbush blueberry, Vaccinium australe.

MUMMY BERRY ON PIN CHERRY: While working on mummy berry control, it was noted several current season shoots on nearby pin cherry, Prunus pennsylvanica L., were blighted. The shoot growth was wilted, resembling frost injury, and was gray in color. Isolations were made and monilioid conidia with disjunctor cells typical for Monilinia vaccinii-corymbosi developed in culture. As far as known, this is the first report of the mummy berry organism naturally infecting pin cherry.

SHOESTRING VIRUS AND FRUIT SYMPTOMS: In 1954, the writer in personal communication with Dr. E. H. Varney, New Jersey Agricultural Experiment Station, was informed of a possible new virus disease of the highbush blueberry. Later, Varney (3) verified his finding that shoestring symptoms were caused by a virus. The Michigan State Department of Agriculture, Division of Plant Industry, was notified and furnished photographs of the symptom expression. In 1956, State plant inspectors tagged bushes for shoestring virus in several blue-

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[1]Journal Paper No. 2172, Michigan Agricultural Experiment Station; Journal Article No. 57-20, Department of Botany & Plant Pathology.

FIGURE 1. Fasciation of primo canes of black raspberry, Logan variety.

FIGURE 2. Fasciation of strawberry peduncles Robinson variety.

berry plantings. The author was able to verify their diagnosis the following season. A survey of these fields disclosed this new virus to be well established. In 14 fields, 416 blueberry bushes were found showing shoestring symptoms.

As only portions of a single cane on a bush may show symptoms of shoestring, it is conceivable that infected bushes are easily overlooked during field inspections. Fortunately, it was noted that Jersey, the major blueberry variety in Michigan, did exhibit a striking fruit symptom not previously described for the shoestring virus. Fruit on infected bushes, even with only one or two canes showing symptoms, develop a premature red to purple cast on the surface exposed to the light. This coloring covers part or all of the upper half of the berry, in contrast to the green fruit on healthy bushes.

BLACK RASPBERRY

FASCIATION -- A FUGITIVE DISEASE: In the course of these studies, black raspberry plants were occasionally found with one or several fasciated canes (Fig. 1). Cross-sectioning of several canes disclosed from three to six individual primo canes coalesced together. In all instances, none of these clones exhibited any visual symptoms of the usual virus infections. Affected plants were tagged and observed for two seasons. None of the fasciated canes were alive the next spring, and no further fasciation was noted on these clones.

STRAWBERRY

ASTER YELLOWS: During the investigation of a severe epidemic of leaf spot on the Sparkle variety in a strawberry field near Keeler, Michigan, in 1957, several plants were observed with the abnormal flower symptoms described for aster yellows (1, 2). The condition was further observed scattered through fields of the Catskill and Premier varieties. Clones of affected plants placed in the greenhouse for indexing failed to survive. One week later, Dr. D. H. Scott, principal horticulturist and Dr. J. R. McGrew, plant pathologist, United States Department of Agriculture, confirmed the diagnosis while visiting the affected fields. This is the first known occurrence of aster yellows virus in strawberry in Michigan.

FASCIATION OF STRAWBERRY: In 1955 and 1956 several clones of the Robinson variety were noted with fasciated peduncles, in addition to more than the usual number of normal peduncles (Fig. 2). The berry borne on these fasciated peduncles also was fasciated, but

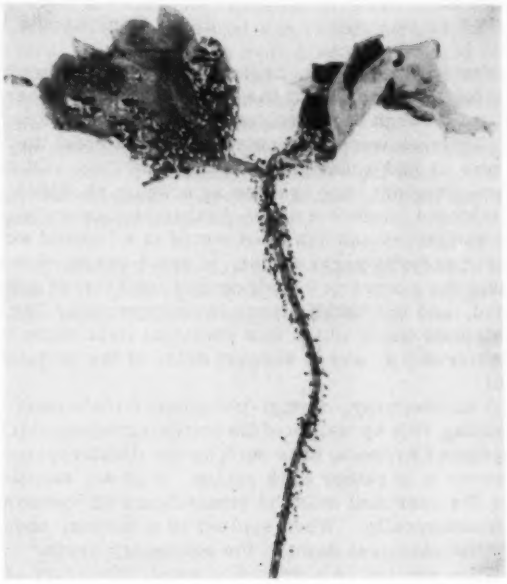

FIGURE 3. Diachea leucopodi on strawberry petioles and leaflets Robinson variety.

ripened normally. Some of these plants indexed to Fragaria vesca (East Malling clone) exhibited only latent C virus, which is commonly isolated in Michigan plantings. These plants were held over in a coldframe. The following season they appeared normal in all respects and a field inspection of the previously affected site disclosed no current peduncle fasciation.

MYXOMYCETES: On several occasions myxomycetes or slime molds have been found on strawberry petioles and leaves (Fig. 3), usually in heavily mulched fields during early summer and late fall following irrigation or rains. In no instances was any apparent plant injury noted. The myxomycetes were identified by Professor E. S. Beneke, Michigan State University, as Diachea leucopodia and Physarum leucopodium.

RED STELE: Several plants of the Fairland variety received for diagnosis showed typical symptoms of the red stele disease caused by Phytophthora fragariae. Examination of the roots disclosed oospores plentifully dispersed throughout the stelar tissue. Since Fairland has been considered a red stele-resistant variety it is assumed that a physiological race pathogenic to Fairland was present in this field. This is the first known infection of Fairland by Phytophthora fragariae in Michigan.

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A LOW-PHYTOTOXIC NEMATOCIDE OF THE ORGANIC PHOSPHATE GROUP

J. R. Christie and V. G. Perry

For several years, beginning in 1949, the writers conducted a small-scale testing program for nematocides at the Central Florida Experiment Station. Chemicals for testing were obtained through the Chemical-Biological Coordination Center of the National Research Council. While we were interested in any material that would kill nematodes, our primary objective was to find a non-phytotoxic nematocide. Of all the chemicals tested, the one that seemed to show greatest promise was an organic phosphate, O-2,4-dichlorophenyl O-O-diethyl phosphorothioate (dichlorophenyl-diethyl-phosphorothioate). This chemical was made available to investigators and has been tested to a limited extent in various parts of the United States. Reports seem to suggest that, in some cases, there may have been a misunderstanding regarding the purposes for which this material is adapted, the methods by which it should be applied, and the results that can be expected. The purpose of this report is to record the circumstances under which this chemical first came to our attention, to note briefly some of its characteristics, and to suggest some of the purposes for which we believe it may prove to be useful.

Dichlorophenyl-diethyl-phosphorothioate usually is applied as an emulsifiable concentrate containing 75% by weight of the active ingredient. It is not as potent a nematocide as some of the halogenated hydrocarbons such as the dichloropropene fumigants and ethylene dibromide, moreover it is rather slow acting. It is not volatile enough to function as a soil fumigant; hence the chemical must be emulsified with water and applied as a drench or mixed with the soil mechanically. When applied as a drench, enough water must be used after application to carry the chemical down to the necessary depth.

When applied as a drench to established turf of Bermudagrass, Cynodon dactylon (L.) Pers., at Sanford, Florida, and of Kentucky bluegrass, Poa pratensis L., at Madison, Wisconsin, a rate of approximately 2 milliliters of the emulsifiable concentrate per square foot resulted in a marked improvement in plant growth and a substantial increase in the spread of the grasses by rhizomes. In both instances, large populations of plant-parasitic nematodes were effectively controlled.

The extent to which a plant may be injured and its growth stunted by a chemical applied to the soil around its roots depends on the kind and age of the plant and many other factors. At the rates required to kill many kinds of nematodes, dichlorophenyl-diethyl-phosphorothioate has a low order of toxicity to most plants under many conditions. It is the least phytotoxic of all the nematocides now available to growers. Germinating seeds and seedlings are more likely to be injured by a chemical than are older plants. This chemical has been applied as a drench at the time some kinds of seeds were planted without adverse effects. In a few instances, germination and growth of the seedlings have been improved. However, results from such applications are variable and somewhat unpredictable, and a waiting period of 1 to 2 weeks between application and planting is recommended.

Dichlorophenyl-diethyl-phosphorothioate is an insecticide of considerable merit and, at rates recommended for reducing nematode injury to turf, it will very effectively control the chinch bug, Blissus laucopterus insularis Barber., an insect of major importance in Florida lawns[1].

This chemical has pronounced residual properties and maintains its nematocidal and insecticidal activity for a long time after application. This residual action has prevented the above-normal buildup of Trichodorus populations which has invariably followed fumigation wherever these nematodes were present.

When chemicals are applied as drenches accurate control of dosage rates is sometimes difficult to achieve. Recommendations for dichlorophenyl-diethyl-phosphorothioate are usually aimed at producing concentrations of 2 to 5 ml of the emulsifiable concentrate per cubic foot of soil. Rates are not very critical, however, and the effects of somewhat greater concentrations in limited areas are not usually serious.

This chemical has little or no effect on nematodes within the roots of living plants. At recommended rates it will slowly but very substantially reduce the numbers of most kinds of nematodes that occur in the soil. It will control root knot (Meloidogyne) by killing the larvae as they hatch, and prevent reinfection of new growth. An encouraging aspect of the work with this material has been the frequency with which its application has been followed by a pro-

[1] Based on experiments by S. H. Kerr, Assistant Entomologist, Florida Agricultural Experiment Stations.

nounced improvement in vegetative growth.

Investigations have failed to disclose any evidence that this chemical is taken up and translocated by plants. When it was first made available, this chemical was recommended for use only on ornamentals. Recently the Food and Drug Administration evaluated the data and is of the opinion that, when applied at recommended rates as a preplanting treatment for certain specified, crops, namely corn, cucumber, pepper, squash, strawberry, and tomato, dichlorophenyl-diethyl-phosphorothioate will contribute no significant residues.

If used intelligently with due regard for its possibilities and limitations, dichlorophenyl-diethyl-phosphorothioate should be a useful material. It provides the home gardener with a means of controlling root knot and other nematode diseases of trees, shrubs, and perennial ornamentals, which has hitherto never been satisfactorily achieved.

AGRICULTURAL EXPERIMENT STATION, UNIVERSITY OF FLORIDA, GAINESVILLE, AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, MADISON, WISCONSIN, COOPERATING

PLANT-PARASITIC NEMATODES FOUND IN ASSOCIATION WITH STRAWBERRY ROOTS IN THE UNITED STATES

Alvin J. Braun[1]

Summary

The findings of a survey of the plant-parasitic nematodes associated with strawberry roots from various sections of the United States are reported. In the northeastern region Pratylenchus spp. were the most prevalent of the parasitic forms. In the southern states Xiphinema, Tylenchorhynchus, and Helicotylenchus were encountered more frequently than other genera. Root knot, while found in all regions, was most prevalent in samples from the north-central region. The incidence of root knot was associated with the source of planting stock. Data presented show that root samples provide a more reliable measure of Pratylenchus spp. population than do soil samples.

INTRODUCTION

In the United States there have been many reports of nematode damage to strawberry roots by Meloidogyne hapla, Pratylenchus spp., Belonolaimus gracilis, and Trichodorus sp. One or more species of the following genera, which have not been established as parasitic on strawberries, have also been reported from the rhizosphere of strawberry plants: Criconema, Hoplolaimus, Paratylenchus (8), Criconemoides (12, 15), Dolichodorus (14), Helicotylenchus (8, 3), Longidorus (12), Trichodorus (7), Tylenchorhynchus (9, 13), and Xiphinema (2, 3). Chapman (5) reported on a survey of plant-parasitic nematodes found in association with strawberry roots in Kentucky, and Horn et al. (10) reported the results of a similar survey in Louisiana.

A survey[2], the findings of which are reported herein, was initiated to determine the distribution of plant-parasitic nematodes associated with roots of strawberry plants in the United States. The presence of certain plant-parasitic nematode species, especially when abundant in a number of the strawberry samples, strongly suggests that strawberry is a suitable host for those nematode species. Therefore, semi-quantitative data of the type presented here should be useful in the investigation of nematodes causing damage to strawberry roots.

MATERIALS AND METHODS

Strawberry root and soil samples were requested from one or more co-operators in each of the 48 States. A total of 231 samples were received from 36 States between August 1 and December 31, 1955. The names of the co-operators[3] and the number of samples sent in by each are given in Table 1.

Sampling kits containing three 1-pint polyethylene bags, labels, data cards and directions for collecting the samples were sent each co-operator. The selection of the sampling site was left to the discretion of the person taking the sample. The following information was requested for each sample: grower's name and address, data collected, approximate planting date, variety, source of planting stock (whether local or out-of-state), the State from which planting stock brought into State within the past 5 years was obtained, soil fumigation (if any), and soil drainage. The samples were made up of portions of roots from each of ten plants selected at random in the sampling area and 20 to 30 gm of soil from the root zone of each plant. The root and soil samples thus collected were placed in a polyethylene bag, labeled, and mailed to

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[1]Temporary appointment as Nematologist, Crops Research Division, Agricultural Research, Service, United States Department of Agriculture, while on sabbatic leave from New York State Agricultural Experiment Station, Geneva, New York, from July 1, 1955 to June 30, 1956.

[2]The writer thanks G. Steiner and A. L. Taylor for their interest and guidance during the course of this survey, and expresses his appreciation to A. C. Goheen for his assistance in planning the survey.

[3]The writer expresses his appreciation to these cooperators whose assistance made this survey possible.

Table 1. Source of samples by regions, States, and cooperators.

Region and State	Collector	Number of samples in survey
Northeastern Region:		
Connecticut	Miller, P. M.	4
Delaware	Braun, A. J., Goheen, A. C.	7
Maine	Goodwin, A., Johnson, E. D.	3
Maryland	Braun, A. J., Goheen, A. C.	10
Massachusetts	Bailey, J. S., Cartwright, C. D., Putnam, J. N.	16
New Hampshire	Rich, A. E.	10
New Jersey	Hutchinson, M. T.	2
New York	Keplinger, J. A., Ruggles, C. E., Van Houten, J. R., Wells, H. D.	16
Pennsylvania	Farr, N. C., Thompson, H. E.	3
Rhode Island	Tarjan, A. C.	3
Vermont	Pratt, L. C.	17
West Virginia	Adams, R. E., Taylor, F. I.	3
Total		94
Southern Region:		
Arkansas	Fulton, J. P.	3
Georgia	Good, J. M.	3
Kentucky	Valleau, W. D.	2
Mississippi	Overcash, J. P., Watson, W. W.	8
North Carolina	Endo, B. Y., Morrow, E. B., Sasser, J. N., Scott, D. H.	6
Oklahoma	Strubble, F. B.	2
Tennessee	Bruer, H. L., Hammett, J. A., Jennings, W. E., Wallaca, B. L.	18
Texas	Norton, D. C.	1
Virginia	Amos, J., Matheny, W. H., Willey, C. R., Williams, A. S.	6
Total		49
North Central Region:		
Indiana	Favinger, J. J., Madinger, F. L., Ulman, P. T.	12
Kansas	Campbell, R. W.	3
Michigan	Bell, H. K., Mullett, C., Trafelet, R.	20
Minnesota	Nelson, G. W., Trampe, W. P.	10
Missouri	Hemphill, D. D.	3
Nebraska	Schuster, M. L.	2
North Dakota	Shultz, J. H.	2
Ohio	Hill, R. G., Winter, H. F.	9
Wisconsin	Gilbert, F. A.	7
Total		68
Western Region:		
Arizona	Milne, R. L., Reynolds, H.	2
California	Bringhurst, R. S., Thomas, H. E.	6
Idaho	Helton, A. W.	1
Montana	Afanasiev, M. M.	6
Oregon	Waldo, G.	3
Utah	Jorgensen, E. C., Thorne, G.	2
Total		20

Beltsville, Maryland for processing.

The nematodes were extracted from a 100-gm portion of each soil sample by means of sieves and Baermann funnels according to the method of Christie and Perry (6). After 24 hours the nematodes from the Baermann funnels were collected in Syracuse dishes, and the total number of nematodes from each sample was determined by counting under the dissecting microscope. Twenty-five nematodes selected at random from each sample were mounted in 5% formaldehyde on a glass microscope slide for identification and counting under the compound microscope. The number of individuals in each genus was then calculated by multiplying by the factor derived by dividing the total number in the sample by the number actually identified.

The root samples were washed thoroughly to remove soil or other debris and were surface-dried by blotting with cloth towels. Portions of live roots, containing lesions when present, were cut into convenient lengths, and a 1-gm representative sample was placed in a standard Petri dish. This sample was carefully examined for lesions, root-knot galls, or other malformations; and then 25 to 30 ml of distilled water was added. The dish was covered and stored at room temperature for 4 to 7 days to allow for the emergence of endoparasitic forms. From counts of the number in each of 25 fields of the dissecting microscope the approximate number of nematodes that had emerged from the 1-gm root sample was calculated by means of a predetermined calibration factor.

For summarizing the results the country was divided into four regions (Table 1).

FINDINGS

Soil Samples

Figure 1 shows the incidence and distribution by regions of the parasitic nematodes most frequently recovered from the soil samples. Paratylenchus was found in low numbers in 4 percent of the samples from the north-central region and Tetylenchus was moderately abundant in 2 percent of the samples from the southern region. Other stylet-bearing nematodes frequently found in the samples from all four regions were Ditylenchus, Psilenchus, Tylenchus, Aphelenchus, Aphelenchoides, and numerous genera of the superfamily Dorylaimoidea.

Pratylenchus was recovered more frequently from soil samples of the northeastern region than in samples from any of the other regions. Meloidogyne occurred most frequently in samples from the north-central region, while Xiphinema, Tylenchorhynchus, and Helicotylenchus were most prevalent in samples from the southern region. Criconemoides, Hoplolaimus, and Trichodorus, although present in a number of samples, were never numerous.

Certain species of several genera were found in samples from widely distributed locations. The genus Pratylenchus was represented primarily by P. penetrans (Cobb), P. pratensis (de Man), and P. zeae Graham. The only root-knot nematode species found was Meloidogyne hapla Chitwood, confirming the findings of Sasser (16). Tylenchorhynchus claytoni Steiner was encountered more often than any of the other species of this genus. The only dagger nematode found was Xiphinema americanum Cobb and the only spiral nematode was Helicotylenchus nannus Steiner.

Belonolaimus gracilis Steiner was not found in any of the samples. No samples, however, were obtained from Florida where, according to Brooks and Christie (4), this nematode is a serious root parasite of strawberries.

Root Samples

The percentage of samples from each region showing various degrees of galling caused by Meloidogyne hapla is given in Table 2. Only half of the samples with root-knot galls showed Meloidogyne larvae and males in their corresponding soil samples. This is to be expected since this nematode is endoparasitic and spends most of its life cycle within the plant roots. Root knot was relatively more common on samples from the northeastern and north-central States. However, infested samples from the southern States usually showed more severe galling.

Root-knot galls were observed on 45 of the 175 root samples for which the immediate source of planting stock was reported. Association of the incidence of root knot with the source of planting stock is shown in Table 3. These findings strongly suggest that in many areas Meloidogyne hapla is a problem on strawberries only when it is brought in on the planting stock. These findings support the observations made in Massachusetts by Bailey (1).

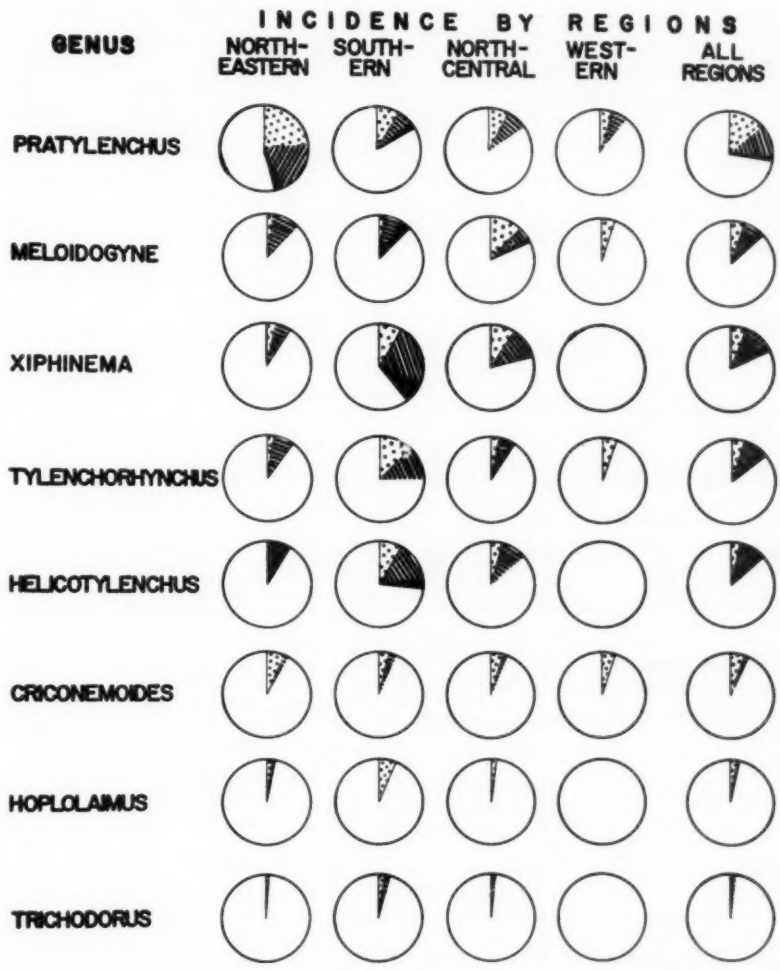

FIGURE 1. Percentage of strawberry soil samples in which certain nematode genera were present in low, medium, and high numbers in each of the four regions of the United States.

Table 2. Prevalence of root-knot galls on strawberry roots from different regions.

Region	Samples examined [a]	Samples with indicated degree of galling			
		Slight	Moderate	Severe	All degrees
	Number	Percent	Percent	Percent	Percent
Northeastern	94	12	6	8	26
Southern	48	2	2	17	21
North-central	65	12	5	14	31
Western	20	0	5	5	10
All	227	9	5	12	25

[a]Collected from August through December, 1955.

Table 3. Relation of incidence of root knot to source of planting stock.

Source of planting stock (Region or state)	Region where planted	Plantings sampled	Plantings with root knot	
		Number	Number	Percent
Northeastern (excluding Delaware and Md.)	Northeastern	40	3	7
	North-central	1	0	
Delaware	Northeastern	10	5	46
	Southern	1	0	
Maryland	Northeastern	21	12	64
	Southern	5	4	
	North-central	7	5	
Southern	Southern	21	2	8
	North-central	2	0	
	Western	2	0	
North-central (excluding Indiana)	Northeastern	1	0	15
	North-central	38	6	
Indiana	North-central	11	6	55
Western	Northeastern	1	0	13
	Western	14	2	
All [a]		175	45	26

[a]Plantings for which source of planting stock was reported.

Table 4. Prevalence of Pratylenchus spp. in strawberry roots from different regions.

Region	Samples examined [a]	Samples with indicated number of nematodes emerging [b]			
		0	1-100	101-1000	More than 1000
	Number	Percent	Percent	Percent	Percent
Northeastern	94	16	19	46	19
Southern	48	35	40	19	6
North Central	65	49	35	14	2
Western	20	55	30	10	5
All regions	227	33	29	28	10

[a]Collected from August through December, 1955.

[b]From 1 gram of washed roots after 4 to 7 days in shallow water in petri dish.

Table 5. Comparison of numbers of Pratylenchus in strawberry roots and in soil.

Population class [a]	Number of samples in each class	Average number of Pratylenchus from		Percentage of Soil samples yielding Pratylenchus
		1 gram of roots	100 grams of soil	
0	75	0	0	0
1-100	66	28	2	21
101-500	49	227	6	47
501-1000	14	782	15	64
1001-2000	15	1,446	20	60
2001-5000	8	2,933	109	100

[a]Based on the number per gram of roots.

The incidence of Pratylenchus spp. in root samples from 227 plantings is given in Table 4. The highest population of Pratylenchus encountered in this survey was 4500 per gram of roots.

The relation between the numbers of Pratylenchus recovered from the roots and from the soil is shown in Table 5. As might be expected, there was a relationship between the number of Pratylenchus per gram of roots and the percentage of soil samples containing Pratylenchus, as well as between the average numbers per gram of roots and per 100 grams of soil. However, it should be noted that where the root population was between 1001 and 2000 only 60 percent of the soil samples contained Pratylenchus. These findings indicate that root samples provide a more reliable index of the presence and prevalence of Pratylenchus spp. than do soil samples.

Pratylenchus was recovered from 56 percent of the samples which showed a trace to 25 percent of rot, as compared with 73 percent for those having 25 to 75 percent rot, and 70 percent for those having more than 75 percent of the roots rotted. Only one sample showed no trace of rot and this sample contained no Pratylenchus. In each of the indicated categories, the average numbers of Pratylenchus extracted from the root samples were as follows: trace to 25 percent rot -- 276 per gm; 25 to 75 percent rot -- 306 per gm; more than 75 percent rot -- 369 per gm. These data indicate a tendency for Pratylenchus to be more prevalent in roots showing a greater incidence of rot. However, only 67 percent of the roots showing rot actually contained Pratylenchus, suggesting that decayed roots do not necessarily indicate the presence of this genus.

Nematode findings were apparently not related to variety, age of planting, or soil drainage. The number of samples from plantings in fumigated soil was too small to permit a determination of the effectiveness of the treatments, especially in view of the fact that the nematode status of the planting stock used in these plantings was unknown.

DISCUSSION

From the findings of this survey it would appear that Pratylenchus is the most prevalent plant-parasitic nematode in association with strawberry roots in the United States. This genus, being an endoparasite, has undoubtedly been introduced into practically all commercial strawberry-producing areas through planting-stock brought in from infested areas. Since Pratylenchus spp. are capable of survival and reproduction on a wide range of host plants, as shown by Jensen (11), it is likely that Pratylenchus spp. have remained in most of the areas where they have been introduced. However, large populations of these nematodes probably exist only where suitable host plants are grown and where soil and other environmental factors are especially favorable for their continued development.

Meloidogyne hapla, although found in samples from all parts of the country and present in more plantings than any one of the ectoparasitic nematodes, appears to be somewhat more limited in its present natural distribution. The evidence associating the majority of the root-knot cases with the source of planting stock suggests the possibility of effectively controlling root knot in many areas simply by planting stock known to be free of root-knot nematodes.

Reference has already been made to the pathogenicity of Pratylenchus and Meloidogyne. In a greenhouse test the pathogenicity of Xiphinema diversicaudatum to strawberries was recently established (18). In view of their frequent association with strawberry roots, Tylenchorhynchus, Helicotylenchus, Criconemoides, Hoplolaimus, Trichodorus, Paratylenchus, Tetylenchus, and other species of Xiphinema should be regarded as potential parasites of strawberry plants.

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THE EFFECT OF NEMATODES ON AZALEAS

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Abstract

Tylenchorhynchus claytoni Steiner, Trichodorus christiei Allen, Tylenchus sp.[2] and Ditylenchus sp.[2] are often found around poorly growing azalea plants in southern California. T. claytoni was the only species that caused a stunting of azalea plants in a greenhouse test.

Numerous azalea plants from commercial growers and home plantings in southern California have been found to harbor a number of species of possible plant parasitic nematodes. Many of these plants exhibited chlorosis, dieback and stunting. The number of samples examined and nematodes found are shown in Table 1.

Table 1. Azalea samples and number of times nematodes found.

Total number samples	Tylenchorhynchus claytoni	Trichodorus christiei	Tylenchus sp.	Ditylenchus sp.	Helicotylenchus erythrinae
60	34	27	25	17	6

Previous records in the literature on nematodes associated with poor growth of azalea include Xiphinema americanum Cobb from southeastern United States (1), Hoplolaimus uniformis Thorne from Rhode Island (3), and Tylenchorhynchus claytoni Steiner and T. dubius (Bütschli) in Maryland (2).

The four species of nematodes most commonly found around azalea roots were introduced into pots in which young azalea plants were growing to ascertain if they affect the growth of azalea.

MATERIALS AND METHODS

Forty-two 6-month-old rooted azalea cuttings (Rhododendron sp., variety Sweetheart) were potted into 5-inch clay pots with new peat moss. One week after planting the lots were divided into six series of seven pots each. The first series received 200 Trichodorus christiei per pot; the second series 200 Ditylenchus sp. per pot; the third series 200 Tylenchus sp. per pot; the fourth series, 200 Tylenchorhynchus claytoni per pot; the fifth series, 50 cc per pot of the washings from which nematodes obtained from azaleas had been screened out by passing through a 325-mesh screen five times; and the sixth series 50 cc per pot of distilled water.

Individual nematodes were picked out under a dissecting microscope with a bamboo splinter and put in 50 cc of distilled water which was poured around the base of each plant. The nematodes were obtained by washing peat moss from a nursery in which poor appearing azaleas had been growing, through screens and placing the washings on a modified Baermann funnel. Pots were randomized on a greenhouse bench.

One year after infestation of pots, the tops of all the plants were cut off and their dry weight taken. Two 50 cc samples of peat moss from each pot were put on a modified Baermann funnel for 3 days and the nematodes were counted. Portions of root systems from each series were cultivated for fungi.

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[2] Appear to be undescribed species.

RESULTS

All the plants grew well during the course of the experiment. The plants growing in soil infested with Tylenchorhynchus claytoni appeared to be slightly behind the other plants in growth after 6 months. After a year the plants in the Tylenchorhynchus claytoni series were noticeably smaller than the plants in the other five series. No obvious differences were observed between the plants infested with the other nematodes and the nematode-free plants. Chlorosis or dieback was not seen in any of the plants. The average dry weight of plant tops and nematode counts in each series are shown in Table 2.

Table 2. Dry weight of plant tops and number of nematodes per 100 cc of peat moss in each series. Average of seven replications.

Series	Dry weight in grams	Nematodes per 100 cc
Trichodorus christiei	12.8	0
Ditylenchus sp.	13.2	0
Tylenchus sp.	11.6	13
Tylenchorhynchus claytoni	5.1**	121
Washings	11.5	0
Untreated	14.5	0

**Significant at 1% level.

The plants in the Tylenchorhynchus claytoni infested series had noticeably smaller root systems than the plants in the other series. Dry root weights were not taken because the root systems were embedded in peat moss.

Large numbers of Tylenchorhynchus claytoni were recovered at the termination of this test from all the pots infested with this nematode. The Tylenchus sp. was recovered from only three of the seven replications. No nematodes were recovered from the Trichodorus christiei or Ditylenchus sp. infested pots.

No pathogenic fungi were obtained from the roots of plants in any of the series[3].

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ANTHRACNOSE OF PANSY AND VIOLET CAUSED BY COLLETOTRICHUM VIOLAE-TRICOLORIS[1]

P. J. Lloyd and D. F. Crossan[2]

Summary

Colletotrichum violae-tricoloris is occasionally destructive to pansy and violet. Lesions on leaves are circular and often zonate; on sepals, petals, stems, and petioles, they are elongate and tan to dark brown in color. The pathogen forms acervuli with setae and one-celled, hyaline conidia averaging 20.6 by 3.8μ in size. The conidia germinate and form appressoria. Penetration occurs most often at the juncture of epidermal cells and the mycelium develops intercellularly. The fungus grows between 10° and 34° C, with an optimum at 29°. Maneb and zineb fungicides at 2 pounds per 100 gallons of water, applied as protectants, will control the disease. Of eight varieties of pansy and two of Viola cornuta, only Cornuta Chantreyland, a viola variety, showed marked resistance.

Little work has been done on Colletotrichum violae-tricoloris R. M. Sm., causal agent of anthracnose of pansy and violet. Although the disease is not of major economic importance, it can cause considerable damage to cultivated pansies and violets. The present investigation was undertaken to add to existing knowledge of the disease, its causal organism, and control.

Pansy anthracnose was first reported by Smith (5) in 1899 in Massachusetts. Since that time it has been reported from many States in the United States (8) and from the Island of Thomas in the Gulf of Guinea (2).

THE DISEASE

Signs and Symptoms

Westcott (8) recorded the symptoms as dead spots with black margins on the leaves, spots on the flowers, with subsequent retardation of floral development, and, occasionally, death of entire plants.

Stevens (6), stated that the leaf spots were first orbicular and definite, later confluent and irregular, and pale-yellow in color; also, blossoms were often deformed.

Dodge and Rickett (3) reported the symptoms as a browning or blotching of the leaves, with dead areas marked by distinct black margins; abnormal development of parasitized petals and death of plants when infection was severe.

According to Tompkins and Hansen (7), pansy leafspot, caused by Cercospora (Centrospora) acerina (Hartig) Tompkins & Hansen has symptoms similar to pansy anthracnose.

The following symptoms were observed under greenhouse conditions, following artificial inoculation.

1. On the Leaves -- The lesions appeared at first as small water-soaked areas about 1 mm in diameter, with a brown to black spot in the center. The lesions enlarged to about 3 mm, became more or less circular, and had tan necrotic centers with dark margins. On heavily infected leaves the lesions often coalesced and formed areas up to 10 mm in diameter that turned brown and necrotic. Concentric zonations often occurred in these areas.

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2. On the Sepals and Petals -- Small, dark brown, elongated lesions surrounded by a light halo appeared on the sepals. On the petals the lesions were dark brown in the center, with lighter shades of brown progressing out to the lesion margin.

3. On the Stems, Petioles, and Flower Stalks -- Sunken, elongated, tan to dark brown, water-soaked areas were characteristic of stem, petiole, and flower-stalk lesions. Pinching of the petioles and flower stalks at the point of origin of the first lesion was common, and progressive dying of tissue down the petiole and stalk often followed.

THE CAUSAL ORGANISM

Cultural Characteristics

The organism was grown on potato-dextrose agar, potato-dextrose-sucrose agar, lima bean agar, and oatmeal agar. These media were prepared according to the methods outlined in Riker and Riker (4).

On potato-dextrose agar and on potato-dextrose-sucrose agar the mycelium of the organism appeared charcoal in color at the center of the culture, progressing to an olive-green at the colony edge. On lima bean agar, the periphery of the colony appeared pink in color.

Sectoring occurred frequently on all types of media. The sectored areas consisted of vegetative rather than sporulating growth.

Morphology

Mycelia from the same culture had a wide range of characteristics, from septate, hyaline, branching hyphae to septate, yellowish-brown, knobby hyphae.

Acervuli were numerous and often confluent, both in culture and on infected plants. Dark-brown setae, which tapered gradually to a point, formed along the acervular margins. They were either single, in pairs, or in triplicate. The setae on infected plants varied from 20 to 70μ in length; those in culture ranged from 115 to 182μ, with the average setae being 145 x 7μ in size.

The conidia of C. violae-tricoloris are oblong or slightly curved with rounded or blunt ends, continuous, hyaline, and granular with vacuoles. They were found to average 20.6 x 3.8μ in pure culture, which is in close agreement with previous reports of an average of 20 x 5μ. The conidiophores are short and hyaline.

Physiology

1. Growth and Sporulation on Artificial Media -- The organism grew well on potato-dextrose, potato-dextrose-sucrose, and oatmeal agar but poorly on lima bean agar. Sporulation was erratic on the first three media; no sporulation occurred on lima bean agar.

Since the organism sporulated well on pansy leaves, an immersed-leaf medium was tested. Two types of this medium were employed. For one type, one or two pansy leaves, depending on size, were placed in a test tube so that their petioles were immersed in water. For the second type, one or two pansy leaves were placed on an agar surface in a Petri dish. Both types were autoclaved for 20 minutes at 15 pounds pressure and 121° C. Small mycelial transfers were made to the leaves, and the cultures were incubated at 25° C.

Sporulation occurred within 7 days on the water-immersed leaves and within 5 days on the sterilized leaves placed on the agar surface.

2. Effect of Temperature on Colony Growth -- Constant-temperature chambers were used to determine the effect of temperature on the growth of C. violae-tricoloris on PDSA. A No. 3 cork borer was used to cut a plug of mycelium that was then transferred to the center of the agar plate. The experiment was replicated four times for each temperature. The test was repeated four times.

The diameter of growth was measured at the end of 2 weeks. The fungus grew well at temperatures ranging from 20° to 32° C, with the optimum for growth occurring at 28°. At 12° and 34° there was very little growth; at 10° and 40° no growth occurred in a 2-week period.

3. Effect of Hydrogen-ion Concentration on Growth in Culture -- The influence of pH was determined in a buffered potato-dextrose broth. Thirty-five ml was pipetted into each of 38 125 ml-Erlenmeyer flasks. The flasks were plugged with cotton and autoclaved at 15 pounds pressure for 20 minutes.

Citric acid (2.6 g), glycine (3.8 g), and KH_2PO_4 (3.8 g) were dissolved separately and then mixed together. Distilled water was added until the volume was 100 ml. After autoclaving at 15 pounds pressure for 20 minutes, 2 ml of the buffer was placed aseptically in each of the flasks.

The buffered broth solutions were adjusted to 9 pH levels (pH 2 to pH 10) by the addition of either 0.1N HCl or 0.1N NaOH. A 5-mm mycelial plug then was placed in each flask, except for the checks. Each pH level was replicated four times, with one of the four being a check on initial pH.

The effect of the hydrogen-ion concentration on the mycelial weight of C. violae-tricoloris after 5 days' growth was determined by removing the mats by filtration, after which they were dried and weighed. The favorable range for growth of the organism was pH 4.7 to 6.5, with the optimum at 5.6. Very little growth occurred at pH 2.3 and 8.9.

Entrance of Pathogen into Suscept

Westcott (8) reported that the conidium of C. violae-tricoloris sends out a short germ tube that, on contact with the epidermis, enlarges at the tip into a brown thick-walled appressorium from which a peg-like infection hypha ruptures the cuticle. This was confirmed by observations made of germinated spores on the surface of pansy leaves. A cleared-leaf technique (1) was used.

Drops of water-suspended spores were placed on 10 pansy leaves (one drop per leaf). The plants were placed immediately in a moisture chamber and held at 25° C. One drop-inoculated leaf was picked at each of the following intervals: 12, 14, 16, 18, 20, 22, 36, and 39 hours. Eight-mm discs were cut from the area where the spores were placed, fixed in a mixture of equal parts of glacial acetic acid and 95 % ethanol for 24 hours, cleared in lactic acid, and stained with a dilute mixture of lactophenol and cotton-blue.

It was found by microscopic examination that by 16 hours some of the spores had germinated and formed germ tubes, at the end of which an enlarged appressorium had developed. After 39 hours spores were found to have germ tubes with appressoria and infection threads. In most cases the appressoria appeared to be located close to, or directly over, junctures between epidermal cells, and the infection threads appeared to penetrate intercellularly.

DISEASE CONTROL

Fungicides

Dodge (3) recommended Bordeaux mixture as a fungicide for control of the pansy anthracnose, but recognized that the copper caused injury to the plants.

We compared several fungicides in greenhouse tests. The materials and rates used were as follows: maneb (Manzate, 2-100), zineb (Dithane Z-78, 2-100) and captan (Captan 50W, 2-100). In a preliminary test, Bordeaux mixture (3-6-100) and Puratized Agricultural Spray (1 pint-100) were eliminated because of severe phytotoxicity.

Two applications of the maneb, zineb, and captan fungicides were applied to potted pansy plants at 5-day intervals. Following the second application, a spore suspension of C. violae-tricoloris was sprayed onto the plant foliage and the pots held in a moist chamber for 48 hours. The plants were then placed on a bench, in a randomized block design, and held for disease development (Table 1).

Resistance

The plants included in this test were inoculated by the same procedure as for the fungicide test. After inoculation they were kept in a moist chamber for 43 hours. Moderate resistance to C. violae-tricoloris was present in some of the pansy varieties tested (Table 2). One viola variety, Cornuta Chantreyland, was highly resistant.

Table 1. The effectiveness of 3 fungicides for control of Colletotrichum violae-tricoloris.

Treatment :	Concentration[a] (Pounds per gallon):	Infection[b] (Percent)
none	-----	62
Captan	2-100	38
Maneb	2-100	18
Zineb	2-100	21

[a] Amount of formulated product
[b] L.S.D. 0.01 = 29.3

Table 2. Relative susceptibility or resistance of several varieties of pansies and violets to Colletotrichum violae-tricoloris.

Variety	Reaction[a]
Pansies	
Giant Swiss - Coronation Gold	S
Giant Swiss - Alpine Glow	M R
Giant Swiss - Raspberry Rose	S
Giant Swiss - Rhinegold	M R
Swiss Giant - Mixed	M R
Dwarf Swiss Giant - Mixed	S
Oregon Giant	S
Jumbo Moon Moth	S
Violets	
Johnny Jump Up	M R
Cornuta Chantreyland (Apricot)	V R

[a] Where V R = very resistant; M R = moderately resistant; and S = susceptible.

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DELAWARE AGRICULTURAL EXPERIMENT STATION

BLOSSOM-END ROT OF CHINESE CHESTNUTS

Marvin E. Fowler[1] and Frederick H. Berry[2]

After the chestnut blight had destroyed the commercially important stands of American chestnut, creating a great void in the forests of the Eastern States, blight-resistant species were sought for possible use in ornamental, wildlife, nut orchard, and forest plantings. One course tried was the introduction of Asiatic chestnuts.

A few Chinese chestnut seeds were introduced into this country at least 60 years ago. Larger quantities were brought in during the middle and late 1920's. Soon after the trees grown from these later introductions began to bear nuts, it became apparent that the Chinese chestnut (Castanea mollissima) had promise of becoming a commercially valuable nut tree in the United States, especially in the South.

But at the same time, serious losses from nut decay were observed at a number of localities. Decay of nuts at harvest time was especially severe in an orchard of pure Chinese chestnut trees at the U. S. Plant Introduction Garden near Savannah, Georgia. The concentration of a large number of trees in this orchard and the severity of the nut decay made this a good place to conduct research on the problem.

The trees in the Savannah chestnut orchard were grown from seed obtained near Nanking, China, in 1924. Several years later these trees began to bear nuts, many of which were decayed at the time of harvest. On a few trees every nut was infected. In 1939, experiments were initiated to determine the agent or agents responsible for decay, extent of decay, relative susceptibility of individual trees, and possible methods of control.

SYMPTOMS

The first symptoms observed, usually 2 or 3 weeks before the chestnuts fully matured, were small brown spots on the green burrs. On most affected burrs, the spots were about the size of a dime and centered around the stylar opening. Later in the season the disease became more advanced and the entire burr turned brown, differing little in appearance from normal mature burrs.

Most nuts affected began to decay at the blossom end. In some, the only symptom of the disease was a very small blackened spot on the shell, usually around the style. The kernels of some nuts were unaffected, but those of others had begun to decay. In more advanced stages, the entire blossom end or all of the shell was discolored, and part or all of the kernel was decayed (Fig. 1). Later in the season, when the shells normally turned brown, the blossom end of the decayed nuts turned black and frequently appeared to be covered with a light-greyish mold.

CAUSAL AGENT

The fungus Glomerella cingulata was consistently isolated from the burrs, shells, and kernels. The production of typical decay from inoculations with the fungus and subsequent reisolation proved that it caused the chestnut blossom-end rot.

The same fungus was later found to cause a large leaf spot of Magnolia grandiflora. It is also known as the cause of bitter rot of apples; and strains of the fungus are known to occur on many unrelated plant species. Cross inoculations made on chestnuts, magnolia leaves, and apples with fungus isolates from decayed chestnuts, apple bitter rot, and magnolia leaf spots proved the isolates to be identical (Figs. 2, 3, and 4). The same fungus was found to be causing part of the nut decay in several other chestnut orchards in Georgia and Maryland. But prevalence of the fungus and the severity of the rot produced was much greater near Savannah than at the other places.

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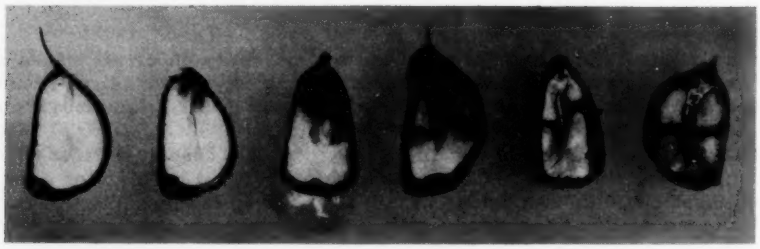

FIGURE 1. Stages of blossom-end rot of chestnut. Normal sound nut on the left. The others show, from left to right, stages from incipient decay to advanced decay. About 3/4 natural size.

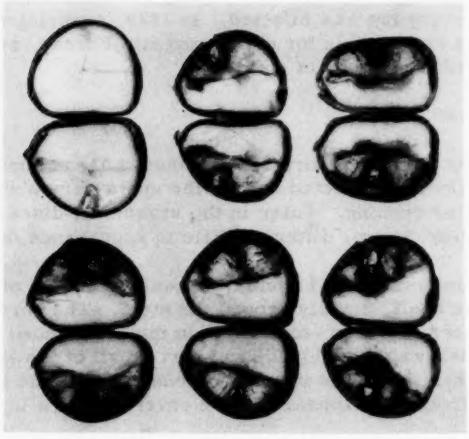

FIGURE 2. Cross inoculations of Glomerella cingulata on chestnuts. Upper left, inoculated with sterile potato-dextrose agar. Upper center, inoculated with an isolate from chestnut that had been used to infect magnolia leaves and reisolated. Upper right, isolate from magnolia leaves that had been used to infect magnolia leaves and reisolated. Lower left, isolate from chestnut. Lower center, ioslate from magnolia leaves. Lower right, isolate from apple. Photographed 3 weeks after inoculations. About 3/4 natural size.

FIGURE 3. Cross inoculations of Glomerella cingulata on apple of the Delicious variety. Clockwise from upper right, inoculations made with isolates from fruit of Ribes, from apple, from chestnut into magnolia leaves and reisolated, from magnolia, from chestnut into apple and reisolated, from chestnut, and with sterile potato dextrose agar. Photographed 5 days after inoculations. About 3/4 natural size.

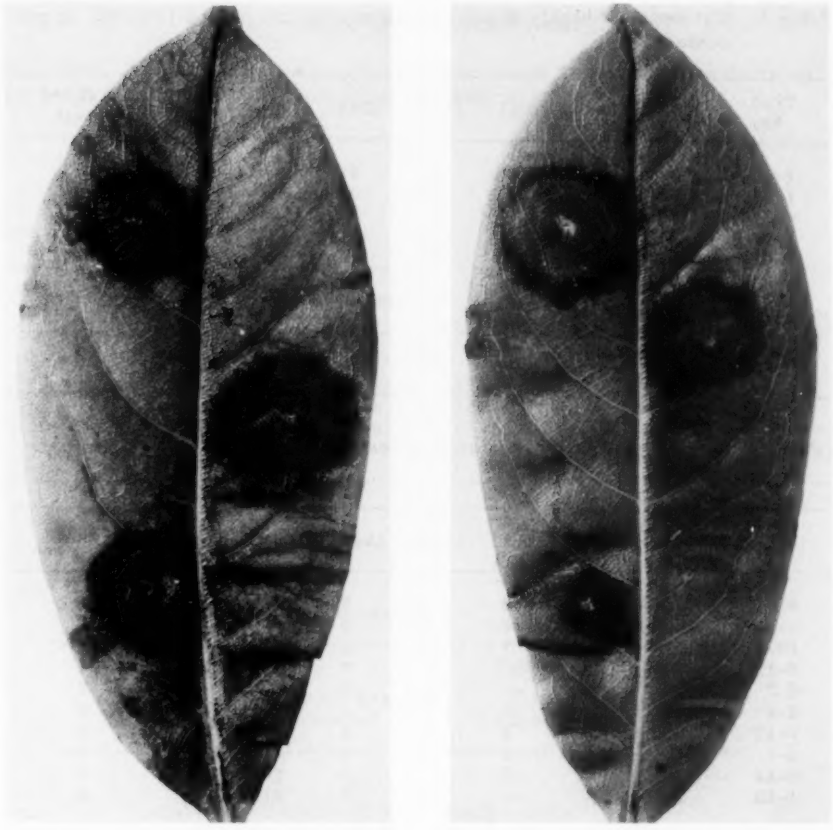

FIGURE 4. Cross inoculations of Glomerella cingulata on magnolia leaves. A -- inoculated with an isolate from chestnut; B -- inoculated with an isolate from magnolia. Photographed 20 days after inoculation. About 3/4 natural size.

EXTENT OF DECAY

From the fall of 1939 through 1945, nut samples were collected from each tree in the Savannah orchard at harvest and were examined for nut decay. It soon became evident that certain trees in the orchard usually had a high percentage of nut decay, while certain other trees usually had a low percentage. Table 1 shows the percentage of nut decay (by years) for the ten trees that had a weighted average of over 35 percent nut decay.

As previously indicated, some trees were highly resistant to this nut decay. Data for the ten trees that had the lowest average percentage of nut decay over the 7-year period are shown in Table 2.

In this orchard of 96 trees the susceptibility or resistance of a majority of the trees to nut decay varied considerably from year to year. For example, among highly susceptible trees, Tree No. 3-7 varied from 100 percent infected nuts in 1940 to 18 percent in 1943; and among highly resistant trees Tree No. 5-13 varied from 29 percent infected nuts in 1939 to none in 1940.

It was also found that trees exhibiting either high susceptibility or high resistance to decay were scattered throughout the entire orchard. Apparently, susceptibility or resistance to decay is an inherent characteristic of certain trees and not a result of environmental conditions.

Table 1. Nut decay of highly susceptible trees for the period 1939-45, in percentage.

Tree No.	1939	1940	1941	1942	1943	1944	1945	Weighted average
7-11	66	--	84	47	41	56	30	48
1-5	68	62	69	49	35	41	34	46
4-17	33	--	74	42	28	44	43	43
7-10	53	--	--	41	31	55	47	42
3-7	54	100	82	41	18	--	--	41
5-2	92	62	26	42	23	--	--	39
4-10	86	55	53	27	26	39	42	38
4-11	37	74	65	16	32	29	38	36
7-2	47	--	16	39	27	42	35	36
9-14	57	--	--	28	60	52	31	36

Table 2. Nut decay of highly resistant trees for the period 1939-45, in percentage.

Tree No.	1939	1940	1941	1942	1943	1944	1945	Weighted average
8-15	6	19	0	3	3	2	11	5
7-16	5	--	7	5	0	--	--	6
10-5	13	5	7	5	2	4	5	6
5-15	--	--	--	6	7	0	5	6
4-7	--	--	--	--	10	5	4	7
5-9	6	20	3	4	4	9	13	7
5-13	29	0	5	7	4	3	4	7
6-1	11	13	5	5	8	--	--	7
6-12	15	0	1	3	8	16	7	7
4-12	27	--	--	6	7	21	6	8

Table 3. Total amount of nut decay, by years, for the period 1939-45.

Year	Nuts examined	Decayed by G. cingulata
	Number	Percent
1939	28,132	34
1940	3,572	27
1941	21,632	24
1942	41,474	15
1943	64,060	15
1944	18,580	18
1945	56,754	17

During the 7-year period 1939-45, a total of 234,204 chestnuts produced in the orchard at Savannah were sliced in half and the nut kernels were examined. Of these, 44,377 (approximately 19 percent) had the type of decay typical of that caused by Glomerella cingulata. In addition to the typical blossom-end rot of chestnuts, several other decays and a starchy breakdown of nut kernels were observed at the time of harvest. These were relatively unimportant; combined they accounted for only 2 percent loss.

The number of nuts examined each year and the percent decayed by G. cingulata are shown in Table 3.

CONTROL EXPERIMENTS

In 1940, 1941, and 1945 various fungicides were tested to determine their value as protective and eradicant materials in controlling blossom-end rot.

Table 4. Results of spray test, 1940.

Plot No.	Trees	Treatment	Nuts examined	Nuts decayed
	Number		Number	Percent
1	14	Sprayed biweekly, 6/17-9/2	773	13
2	12	Sprayed biweekly, 7/22-9/2	454	32
3	7	Sprayed biweekly, 6/17-7/22	220	57
4	63	Control	2,060	28

Table 5. Results of spray test, 1941.

Plot No.	Trees	Treatment	Nuts examined	Nuts decayed
	Number		Number	Percent
1	23	0.5% Elgetol	7,743	31
2	47	Control	12,666	23

Table 6. Results of spray tests, 1945.

Plot No.	Trees	Nuts examined	Nuts decayed
	Number	Number	Percent
1	9	5,496	14
2	9	7,481	21
3	9	9,954	19
4	10	8,851	21
5	26	24,972	19

In 1940 the chestnut orchard at Savannah was divided into four plots. Three of these plots were sprayed with 6-6-100 Bordeaux mixture at biweekly intervals between specified dates. Table 4 summarizes the results of this test.

The data present conflicting evidence. Although the results obtained by spraying from flowering time until harvest time indicate that the treatment reduced the percentage of decayed nuts when compared with the unsprayed control trees, a further comparison of the control with the trees sprayed through only part of the season would indicate an adverse effect by the fungicide. Even if a degree of control could be obtained by applying six or more spray applications, the amount of decay reduction obtained does not appear sufficient to be economically

feasible in orchard management.

In March 1941, an attempt was made to control this nut decay by means of an eradicant fungicide applied to the ground while the trees were still dormant. Two plots were laid out in the orchard. The ground under Plot No. 1 was sprayed with Elgetol, a commercial preparation containing approximately 30% of sodium dinitro-ortho-cresol, in 0. 5% concentration by volume. Plot No. 2 was left untreated as a control. As shown in Table 5, Elgetol was not effective in controlling the disease.

In 1945, 8-8-100 Bordeaux mixture and Phygon-XL were tested as protective materials. Five plots were laid out in the orchard and these received the following treatments:

Plot No. 1 - 8-8-100 Bordeaux; 4 sprays from July 23 to harvest.
Plot No. 2 - Phygon-XL; 4 sprays from July 23 to harvest.
Plot No. 3 - 8-8-100 Bordeaux; 3 sprays from August 1 to harvest.
Plot No. 4 - Phygon-XL; 3 sprays from August 1 to harvest.
Plot No. 5 - Control

The results of these spraying tests are shown in Table 6.

In three of the four sprayed plots the percentage of decayed nuts was as much as or more than the percentage of decayed nuts in the untreated plot. In Plot No. 1 the percentage of decayed nuts was 5 percent less than in the control plot, but this figure can not be considered significantly lower. The results indicate that these fungicides had little or no effect on the incidence of nut decay.

Since 1945, thinning the stand of trees became necessary in the overcrowded orchard near Savannah. About one-third of the trees were removed to open up the orchard and release trees for more vigorous growth. In thinning the stand, those trees having high susceptibility to the blossom-end rot were discriminated against and were removed. Removal seems to offer the best-known control for the disease, since the Chinese chestnut trees remaining in the orchard now appear to be relatively free of blossom-end rot.

DIVISION OF FOREST DISEASE RESEARCH, FOREST SERVICE, AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

THE EFFECT OF STORING APPLES IN POLYTHENE BOX LINERS ON THE DEVELOPMENT OF BULL'S-EYE ROT, CAUSED BY NEOFABRAEA PERENNANS.[1]

D. L. McIntosh and S. W. Porritt[2]

Abstract

The number of bull's-eye rot infections, caused by Neofabraea perennans, that developed in cold storage in fruit packed in polythene box liners was much higher than that found in fruit wrapped in paper or stored loose. Relative humidity and carbon dioxide concentration were much higher in the atmosphere within the bags than in the storage atmosphere. Bagged fruit, judged by color, firmness and flavor, was less ripe than wrapped fruit. The data indicate that a much higher percentage of fruit from orchards affected by N. perennans may be infected with this fungus than was formerly realized. If fruit used in trials on the control of this disease is stored in polythene box liners after treatment, greater differences between treatments may appear than if the fruit is wrapped in paper or stored loose.

INTRODUCTION

In 1955, tests were made at the Experimental Farm, Summerland, British Columbia, to determine the effect of 1.5 mil polythene box liners on the storage life of Yellow Newtown and Golden Delicious apples. When the fruit was examined after 5 months in cold storage, 25 to 50 percent of the apples stored in polythene were found to be infected with bull's-eye rot, caused by Neofabraea perennans Kienh. (imperfect state = Gloeosporium perennans Zeller & Childs), while only 4 percent of the apples stored loose in boxes were affected by the disease. Furthermore, the percentage of diseased apples was several times greater in lots stored in unsealed than in sealed liners.

These results were not anticipated at the time the samples were collected in 1955 and no records were made of the N. perennans inoculum potential in the trees from which the fruit was taken. However, the samples were well composited before storage and it seemed likely that the increased development of rot in the bagged lots was due to their storage environment. These tests, in the 1956-57 storage season, were planned to obtain additional information on the effect of storing fruit in polythene box liners on bull's-eye rot development.

METHODS

In October 1956, Yellow Newtown apples were harvested from two orchards with a history of bull's-eye rot losses. The fruit from each orchard was composited and divided into two lots. One lot was inoculated immediately before packing with a spore suspension of Neofabraea perennans, and the other lot was stored directly.

The spores used for inoculation were obtained by growing the fungus on pieces of apple leaf, sterilized with propylene oxide, placed on lima bean agar, and inoculated with a drop of spore suspension. Greater production of macrospores was obtained by this means than by growing the fungus on potato dextrose, malt, or lima bean agar, or by placing the leaf pieces either on water agar or on moist filter paper. Spores were washed from the cultures after 7 days, and each fruit was inoculated by swabbing with a cheesecloth pad dipped in the spore suspension.

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[1] Joint contribution from the Botany and Plant Pathology Division, Science Service (Contribution No. 1650), and the Horticulture Division, Experimental Farms Service (Contribution No. 921), Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathologist, Plant Pathology Laboratory, and Pomologist, Experimental Farm, respectively, Summerland, British Columbia.

Samples from both lots were stored immediately after packing at 32°F under the following conditions: 1) in sealed 1.5 mil polythene box liners, 2) in polythene box liners folded but not sealed, 3) in dry wraps in bushel boxes. In addition one box was stored loose with no paper wraps.

The fruit remained in cold storage from October until May 10, except for an examination April 24. It was removed to 70°F storage May 10, and re-examined May 14.

RESULTS AND DISCUSSION

As shown in Table 1 the percentage fruit infected with bull's-eye rot was much higher in all lots stored in polythene box liners than in those stored in paper wraps. These differences increased after the fruit had been in storage at 70°F for 4 days. On May 14 there was no appreciable difference in the percentage of fruit infected in sealed and non-sealed bags. In the samples of fruit not inoculated, the number of infections per infected fruit in sealed liners was 70 percent greater than that in non-sealed liners. In 1955, more infection developed in bags that were not sealed, but the reason for this inconsistency is not known.

Table 1. The effects of inoculation and storage conditions on the development of bull's-eye rot.

Treatment	Percentage infected		Average no. infections per infected fruit
	April 24	May 14	
Inoculated -			
Stored loose	13.2	18.7	1.0
" in paper wraps	9.4	13.5	1.2
" in sealed liners	73.5	95.6	2.7
" in non-sealed "	59.0	90.1	3.0
Non-inoculated -			
Stored in paper wraps	7.1	15.8	1.1
" in sealed liners	35.1	66.2	1.7
" in non-sealed "	40.5	67.1	1.0

Kienholz (3) and Wade (4, 5), in their respective studies on the rots produced by Neofabraea perennans and Gloeosporium album, found that the amount of infection that developed in ripe fruit could be reduced by applying sprays in the orchard at an early stage in fruit development. They concluded that infections may occur at any time during fruit development that weather conditions are favorable, and that these infections remain latent until the fruit reaches a certain stage of ripeness. No evidence that bull's-eye rot may spread from diseased to healthy fruit in storage has been noted in reports of other investigators, nor was there any evidence that it occurred in this experiment. It is assumed, therefore, that all composite lots of non-inoculated fruit possessed about the same number of latent infections, or the same inoculum potential, at the time they were stored, and that more infections developed in bagged fruit than in wrapped fruit because of a more favorable environment within the polythene bags.

The reasons that storage in polythene liners favors the development of bull's-eye rot are not known but the high humidity in the bags probably is of prime importance. The relative humidity of apple cold storage atmospheres usually is 80 to 90 percent, while that in the polythene bags was close to 100 percent. If there are any ungerminated spores on the surface of the fruit at the time it is packed, the moisture content of the air in the bags may be sufficiently high to permit spore germination and infection of the fruit. This hypothesis, if valid, would account for the much higher percentage of fruit infection in the lots that were inoculated before bagging. Edney (1) found that the wastage from N. perennans infections increased

as storage humidities were raised but the variation that occurred through the range of humidities used in commercial storages was not investigated.

In his experiments on the control of this disease, Edney found that storage of fruit in an atmosphere of 5 percent O_2 and 3 percent CO_2 might delay the development of infections to some extent, if the level of natural infection was low. The storage atmosphere in the sealed polythene bags used in our experiment contained about 5.5 percent O_2 and about 6 percent CO_2. In some instances a very slight increase in CO_2 content, of less than 1 percent, was found in folded non-sealed liners.

Kidd and West (2) found that lenticel injury can occur from the accumulation of volatile esters in toxic amounts and that this injury predisposed the affected cells to attack by lenticel-rotting fungi. Dissipation of volatiles from within polythene bags is less rapid than from among apples stored in wraps, but there was no evidence of lenticel injury in any lots of bagged fruit.

Since bull's-eye rot usually does not appear in fruit until some time after it is harvested, it has been accepted generally that apple tissue is able to resist invasion until it ripens, and its resistance probably decreases rapidly as it approaches senescence. When the fruit was examined April 24, that stored in polythene, judged by its color, firmness and flavor, was less ripe than that in wraps. There was a larger number of small lesions in bagged than in wrapped fruit, but the maximum size of lesions in each lot was about the same.

The results of this study indicate that a much higher percentage of fruit from orchards with an inoculum potential of N. perennans may be infected at the time it is harvested than was formerly realized. In the records of 24 years' observations published by Kienholz (3), the percentage of crop repacked because of bull'-eye rot, in the plant under study, only once exceeded 10. The wastage in British Columbia packing houses has been of the same order, but in the 1955 and 1956 tests with fruit stored in polythene, a maximum of 50 percent and 40 percent respectively, was infected. Obviously, a satisfactory control for this disease must be found before it will be practical to store fruit susceptible to bull'-eye rot in polythene liners.

The knowledge that storing fruit in polythene bags favors the development of bull's-eye rot may be useful in future experiments on the control of this disease. Heretofore only small differences have appeared between treatments, with only a small percentage of untreated fruit infected. Storage of fruit in polythene may result in larger differences between treatments and enable their relative efficacy to be assessed more readily.

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APPLE POWDERY MILDEW SPRAY TRIALS AT WENATCHEE, WASHINGTON IN 1957[1]

Roderick Sprague[2]

Powdery mildew [Podosphaera leucotricha] in north-central Washington has recurred in moderately severe quantities after being nearly annihilated by the winter of 1955-56. Although it was still somewhat erratic in distribution in early season in our plots it spread rapidly in unsprayed trees during mid-spring.

The treatments were applied by hand gun, using a stationary spray outfit. As shown in Table 1, sprays were applied at approximately 12- to 14-day intervals, up to a total of six sprays in some cases. Counts of percentage of terminals diseased were made before each spray and on July 15 after the final spray. Estimates were also made on July 15 of approximate leaf surface for each tree covered by powdery mildew. These factors were again classified from 1 (heavy) to 5 (virtually free). Each "percent of mildew" in Table 1 is the average for five replications of 10- to 11-year-old trees, except the checks which are the summary of ten such trees.

The results this year were the most promising obtained in 8 years of work on this problem. The following materials appear to be worthy of further trials in addition to already accepted Karathane: Geigy G28810; Phybam; American Cyanamid Exp. F. 5223; Carbide Carbon Res. F. 10521 and Carbide Carbon Res. F. 9688. Penn Salt P398 was received later in the summer, but at the 2-pound rate showed promise. We have 2 years' results with Geigy G28810, which, from information we have locally, needs expanded trials, as it looks particularly promising. Phybam, which is a mixture of wettable sulfur, Phygon, and ferbam, gave outstanding control; better than sulfur alone. However, it causes mild leaf cast of small leaves and must be used only on sulfur-tolerant trees (Jonathan and Romes). We feel that it will be safe to recommend it at 3 pounds per 100 gallons on such varieties under Wenatchee conditions.

An extensive trial compared standard Karathane and Microcel-carried Karathane at 1 pound rates in conjunction with two kinds of wetting agents at 0, 1, 2, and 4 ounce rates. These trials are not reported in Table 1 except for standard Karathane, used at the 1 pound rate with 2 ounces per 100 gallons of Triton X 114 wetter and Microcel Karathane with no wetter. Both standard and Microcel Karathane gave very good control.

The standard treatment of two early sprays of liquid lime-sulfur at 3 percent, then 2.5 percent, followed by a calyx spray of 3 pounds of wettable sulfur, gave fair control until summer when powdery mildew flared up in this corner of the orchard, and spread to some extent into the Karathane treatment adjacent.

Dyrene was discarded because of injury to the fruit, as was GCEF8. Some combinations and certain materials such as Thioneb were ineffective.

The addition of 8 ounces of Glyodin to Karathane did not indicate that improved control warranted the extra cost. However, this test was close to the corner of the plot where powdery mildew was rampant in late season, therefore further trials with this combination would be desirable. On the other hand, although the block of trees sprayed with Geigy G28810 was within a few feet of this group, it continued free from heavy infection until the end of the experiment.

General Chemical Sticker, used at the 2-ounce rate with Karathane, proved acceptable. A long-shot attempt to prevent infection of trees by using 8 ounces of this wetting agent by itself failed by mid-summer. Its early promise (April 22 to June 2) was likely due to escape from infection in this portion of the orchard.

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[1]Scientific Paper No. 1674, Washington Agricultural Experiment Stations, Pullman, Washington. Project No. 1164.

[2]Plant Pathologist, State College of Washington, Tree Fruit Experiment Station, Wenatchee, Washington.

Table 1. Apple powdery mildew counts in Black Jonathans, Tree Fruit Experiment Station, sprayed at (1) Pre-pink on April 11; (2) Pink on April 24; (3) Calyx on May 7; (4) Cover on May 20; (5) Cover on June 3; (6) Cover on June 15.

Treatment	Rate Per 100 Gals.	When Applied	Per Cent Mildew When Counted On:							b Factor	c Corrected Loss
			Apr. 10	Apr. 22	May 7	May 19	June 2	June 15	July 15		
Lime Sulfur Sulfur	3⅞, 2⅛ 3 lbs.	L.S. - 1,2[a] S. - 3	2.6	2.7	5.1	6.5	7.7	8.2	27.3	2	14.7
Karathane + Triton X 114	1 lb. + 2 oz.	1,2,3,4,5	3.6	4.0	4.6	6.0	6.9	17.1	10.0	3½	2.9
Karathane + Glyodin	1 lb. + 8 oz.	1,2,3,4,5	2.1	2.7	2.5	3.1	3.4	8.9	6.2	4	1.6
Unsprayed Checks	- -	- -	4.7	4.3	6.2	11.5	17.7	27.9	28.3	1½	18.9
Phybam	4 lbs.	1,2,3	3.4	0.9	1.1	0.9	0.9	3.3	5.3	4½	1.2
Dyrene	1 lb.	1,2,3,4,5	0.2	0.5	0.8	0.9	0.2	7.6	10.0	3	3.3
Thioneb	2 lbs.	1,2,3,4	3.2	3.5	4.6	6.9	12.1	Discard	- -	- -	- -
Geigy G28810	1 lb.	1,2,3,4,5,6	0.2	0.5	0.9	0.8	0.9	2.3	3.8	5	0.8
GCEF8	2.5 oz.	1,2,3,4	2.2	0.5	0.7	1.6	0.7	2.8	4.1	3½	1.2
Karathane + GCEF8	1 lb. + 1 oz.	1,2,3,4	0.4	0.2	0.3	0	0.3	0.8	3.6	4	0.9
Thioneb + Karathane	2 lbs. + 1 lb.	1,2,3,4	1.4	0.4	2.1	1.5	2.9	2.5	Discard	- -	- -
Kelthane + Karathane + Triton X 114	1½ + 1 lb. + 2 oz.	Kel. - 1,4 Kar. -1,2,3,4	0.9	0.4	3.5	2.7	3.8	3.9	Discard	- -	- -
Amer. Cyan. Exp. F. 5223	1½ lbs.	1,2,3,4,5,6	0.9	0.3	1.6	1.9	2.5	2.7	2.6	3½	0.7
Karathane + Triton X 114	1 lb. + 2 oz.	1,2,3,4,5,6	1.3	0.5	1.2	1.4	1.7	1.2	1.6	4	0.4
Microcel-carried Karathane	1 lb. no wetter	1,2,3,4,5,6	1.6	1.0	2.3	1.5	3.0	2.3	4.0	4	1.0
Carbide Carbon Res. F10521	1 lb.	1,2,3,4,5,6	2.9	1.0	1.9	1.8	2.0	2.5	5.5	4	1.4
Carbide Carbon Res. F9688	1 lb.	1,2,3,4,5,6	3.1	1.6	2.3	2.4	1.9	2.2	3.2	4	0.8
Karathane + Gen. Chem. Sticker	1 lb. + 2 oz.	1,2,3,4,5,6	0.7	0.8	0.2	0.6	1.1	1.3	2.8	4	0.7
General Chem. Sticker Only	8 oz.	1,2,3,4,5,6	1.0	0.8	0.3	1.7	2.0	6.0	7.5	1½	5.0
Penn Salt P398	1 lb.	5,6	- -	- -	- -	- -	[d] 12.1	7.7	11.3	3	3.8
Amer. Cyan. Exp. F 5223 + Triton X 114	1 lb. + 1 oz.	6	- -	- -	- -	- -	- -	[d] 2.5	3.8	3½	0.7
Penn Salt P398	2 lbs.	6	- -	- -	- -	- -	- -	[d] 3.9	2.6	4	0.7

[a]The numbers refer to sprays listed and dated in title of table: i. e., 1 = pre-pink applied April 11.
[b]Factor (numbers 1 to 5) shows surface covered by mildew, the less surface covered, the higher the number.
[c]To obtain corrected loss, divide percent loss on July 15 by the factor.
[d]Before spraying.

EFFECTS OF AN ETHYLENE DICHLORIDE AND TRICHLORETHANE MIXTURE ON THREE CITRUS FRUIT PATHOGENS

S. Z. Berry[1]

Abstract

Quantitative and qualitative effects of a mixture of ethylene dichloride and trichlorethane gas (Sound Delivery Vapor) on three citrus fruit pathogens, Diplodia natalensis, Phomopsis citri, and Penicillium digitatum, were studied. Germination of the spores of the three pathogens was inhibited when they were exposed in vitro to the gas for 6, 10, 20 and 30 hours, but the effect of the gas was rapidly lost on aeration. No spores of the last two species germinated while they were in contact with the gas. A 16-hour exposure of oranges to the gas was only slightly more effective than a 6-hour exposure in controlling fruit decay. The decay-inhibiting effect of the gas was lost rapidly in the second week after treatment.

The principal post-harvest disease problem of Florida citrus is stem-end rot, caused by Diplodia natalensis Pole-Evans and Phomopsis citri Fawc., and green mold rot, caused by Penicillium digitatum Sacc. D. natalensis is most prevalent during the fall. Phomopsis citri and Penicillium digitatum predominate during the winter and spring. A mixture of ethylene dichloride (63.52%) and trichlorethane (35.48%) called Sound Delivery Vapor[2] is being used commercially in Florida as a fumigant for the control of post-harvest decay of oranges. Winston[3] has found it to be highly variable in its effectiveness as an inhibitor of decay of oranges due to these three fungi. This study was therefore undertaken primarily to determine the effect of this gas mixture on the decay organisms in vitro. In addition, a small-scale test was carried out to study its effect on the decay of naturally infected orange fruits.

MATERIALS AND METHODS

Isolates of the three pathogens were obtained from diseased oranges collected in the field. Diplodia natalensis sporulated better on steamed stems of coffee weed (Ditremexa spp.) than on several other media used, such as steamed wheat, oats, oat straw, and orange twigs. On the coffee weed medium single-celled hyaline spores and double-celled pigmented spores (transformations of the hyaline spores) were both produced abundantly. Phomopsis citri also sporulated well on the coffee weed. Penicillium digitatum spores were taken from cultures maintained on potato-dextrose agar.

In preliminary tests the mycelial growth rates of the three pathogens studied were not measurably altered by exposure to the gas for 6 hours, but the treatment had an inhibiting effect on spore germination. Consequently a study of the latter effect was made. Spore suspensions were prepared in sterile tap water. Drops of the suspension were then placed on agar plates, and any excess moisture was drained off. The plates were exposed to the gas mixture at the commercial dosage of 3.4 cc per cubic foot in a chamber maintained at 83° F. For exposure periods greater than 6 hours the plates for each treatment were placed in open 5-gallon jars in the chamber, and at the end of the first 6 hours the jars were closed. At the end of the specified treatment period the jar was aerated. After fumigation the plates were kept at 75° F. At least 100 spores were counted per treatment.

The effect of the gas on naturally infected fruit was demonstrated with oranges freshly

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[1] Plant Pathologist, formerly Agricultural Marketing Service, United States Department of Agriculture, Orlando, Florida; now Agricultural Research Service, Beltsville, Maryland. The writer expresses his appreciation to J. R. Winston for his suggestions.

[2] Product of the Brogdex Company, Orlando, Florida.

[3] Winston, J. R., United States Department of Agriculture, Orlando, Florida: Personal communication.

picked, washed, and dried. The fruits were exposed to the gas mixture (3.4 cc per cubic foot) in a 377-cubic foot chamber maintained at 83° F. For comparison, oranges were treated with a 2% solution of Dowicide A-Hexamine, another commercially used citrus decay inhibitor; the fruits were dipped in the solution for 3 minutes, rinsed with water, dried, and then stored at 70° F.

RESULTS

Preliminary tests were carried out in February and March 1957, to determine the effect of the gas mixture on germination of spores of the three pathogens. In these tests the spores were exposed to the gas for 6 hours. This treatment prevented the germination of spores of Phomopsis citri and Penicillium digitatum, while germination of Diplodia natalensis spores remained high. In all cases germination of the treated spores increased sharply 4 hours after aeration, and after 16 hours it approximated that of the untreated spores. Consequently, the action of the gas was believed to be fungistatic. Certain other gases used for citrus decay control have been shown to be fungicidal (1, 2). The effect of the gas mixture on spore germination was also studied for exposure periods of 10, 20, and 30 hours (Table 1). The action remained fungistatic during the maximum exposure period of 30 hours. After the plates in the several treatments were aerated for 16 hours, germination equalled that of the controls. The only exception was the treatment in which P. digitatum spores were exposed to the gas for 30 hours, in which case there was still only 72 percent germination after 16 hours of aeration.

Table 1. Percent germination of spores of three citrus fruit pathogens on agar plates exposed for periods of 6 to 30 hours to Sound Delivery Vapor gas at 83° F, June 4, 1957, and of untreated spores in equal periods of time[a].

Pathogen	Percent Germination							
	Treated spores after indicated exposure time[b]				Controls (untreated) spores in indicated number of hours			
	6 hours	10 hours	20 hours	30 hours	6 hours	10 hours	20 hours	30 hours
Diplodia natalensis								
Hyaline spores	87	92	87	89	100	100	100	100
Pigmented spores	32	34	80	79	95	95	97	97
Phomopsis citri	0	0	0	0	21	92	92	95
Penicillium digitatum	0	0	0	0	3	27	79	95

[a] At least 100 spores were counted per treatment (four preliminary tests gave similar results).

[b] After aerating at 75° F for 16 hours, the spores exposed to the gas had a percentage germination approximately equal to that of the 30-hour control (see text).

Germination of D. natalensis spores was inhibited less than that of spores of the other pathogens, and both hyaline and pigmented spores germinated even while exposed to the gas. Germination of pigmented spores was less than that of hyaline spores; however, after exposure to the gas for 20 and 30 hours the germination of the pigmented spores approached that of the hyaline spores. Many of the hyaline as well as the pigmented spores that germinated after exposure for 6 hours showed abnormal, many-branched, rudimentary germ tubes; and in addition many of the hyaline spores had swollen germ tubes (Fig. 1). Diphenyl has been shown to cause similar types of germ-tube abnormalities (3). The abnormal germ tubes made very little further development while exposed to the gas. For example, the germ tubes of pigmented spores which had germinated after 6 hours of exposure to the gas averaged 19.3μ in length, while those of spores exposed for 20 hours averaged 33.0μ; the control averaged 285.5μ. After aeration for 4 hours the germ tubes of spores exposed to the gas for 6 hours averaged 225.0μ, and of those exposed for 20 hours 85.6μ. In the same period the germ tubes of control spores averaged 1,025.0μ. Each germ-tube-length value is an

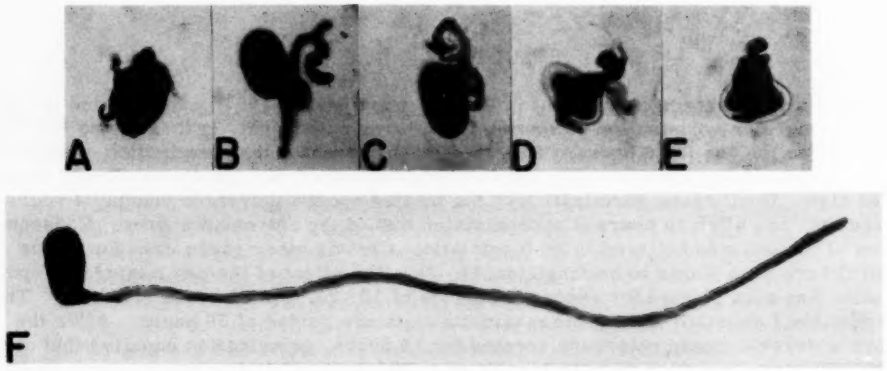

FIGURE 1. A - E, Abnormal germ-tube development of Diplodia natalensis spores while exposed to Sound Delivery Vapor gas: A, Pigmented spore after 6-hour exposure; B and C, pigmented spores after 10-hour exposure; D and E, hyaline spores after 10-hour exposure. F, ungassed (control) germinating spore after 6 hours. All X about 337.5.

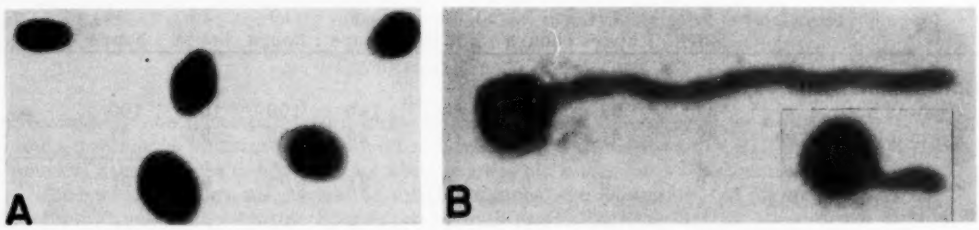

FIGURE 2. A, Spores of Penicillium digitatum, which swelled but did not germinate during a 10-hour exposure to Sound Delivery Vapor gas. B, Ungassed (control) germinating spores after 10 hours. Both X about 1350.

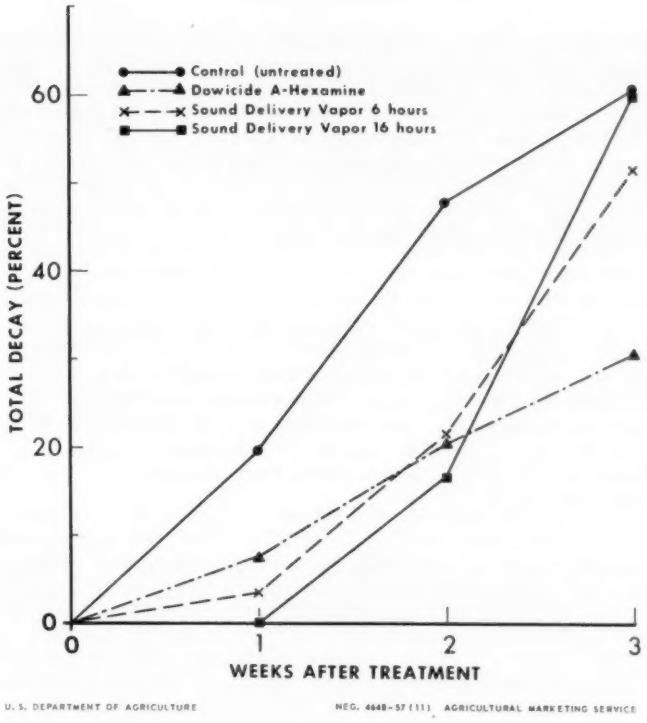

FIGURE 3. Total decay due to stem-end rot and green mold of Pineapple oranges treated with Sound Delivery Vapor gas (3.4 cc per cu. ft.) and 2% Dowicide A-Hexamine dip. One test with an average of 50 fruits per treatment. Stored at 70° F.

average of 40 measurements. Thus the germination of spores subjected to the longer exposure showed some residual inhibition. However, after 16 hours of aeration germ-tube growth of spores for all treatments increased and was characterized by branching and initiation of mycelial growth.

Germination of P. citri and P. digitatum spores after exposure to the gas mixture was markedly inhibited and no germination occurred during exposure. Swelling of P. digitatum spores without accompanying germination became distinct after exposure for 10 hours (Fig. 2), and became more pronounced with lengthened exposures. P. citri spores also swelled during the longer gas exposures, and none germinated until the cultures were aerated. After spore germination was activated, the rate of germ-tube development of these pathogens was similar to that of D. natalensis following aeration.

An experiment was conducted to study the effectiveness of Sound Delivery Vapor gas in the control of decay on Pineapple oranges on March 5, 1957. An average of 50 oranges was included in each treatment. Exposure to the gas for 6 hours reduced decay for 1 week (Fig. 3). However, in the second and third weeks decay was not materially decreased as compared with that of the untreated lots. For comparison, Dowicide A-Hexamine treatment was used. Although not so effective as the gas in controlling decay the first week, this material maintained its decay-inhibiting properties over a longer period. Control of stem-end rot and green mold decay in the 16-hour gas treatment was not materially better than the

6-hour treatment 2 weeks after treatment. Green mold was the prominent decay in the 16-hour exposure treatment, while stem-end rot was more prominent in the 6-hour exposure.

CONCLUSIONS

Sound Delivery Vapor gas had an inhibitory effect on germination of spores of Diplodia natalensis, Phomopsis citri and Penicillium digitatum in contact with it. Spores of P. citri and P. digitatum did not germinate while in contact with the gas. Exposure of the pathogens to the gas for periods in excess of those used commercially did not reduce the viability of spores as determined by germination subsequent to aeration, but did result in a longer period of inhibition. A longer period of gas exposure improved decay control only slightly as compared with the shorter exposure, and the decrease in control of decay was rapid 1 week after fruit treatment. The short fungistatic life of this gas indicates that its use should be based on repeated fumigations during long storage periods.

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CHARCOAL ROT ON STRAWBERRY IN ILLINOIS

Bill Tweedy and Dwight Powell

The charcoal rot organism, Botryodiplodia phaseoli (Maubl.) Thirumalachar (4), was isolated from vascular tissue of both roots and above-ground parts of Dixieland strawberry plants collected from the Centralia area of south-central Illinois, September 29, 1957. Infected plants showed symptoms of wilting, dwarfing, and eventual death. The mother plant, set the previous spring, usually manifested the most definite symptoms. In many cases, however, new plants that developed after renovation were infected.

While strawberry is listed (5) as one of 284 hosts of the charcoal rot fungus this is the first time that it has been isolated from strawberry in Illinois. Previously it has been reported as a pathogen of corn (3) and soybean (1) in Illinois. With the recent expansion of the strawberry industry, in this part of the State, to land that had been used primarily for corn and soybean production, this disease may become a serious problem. Thus far, its importance on strawberry has not been fully evaluated. It was found to be generally distributed in many plantings, and it will definitely cause a reduction in yield in 1958.

It has been established (2) that the pathogen causing charcoal rot requires a high temperature along with a dry soil for best development, which was the situation that existed in this area during August and September of this year. It may be possible to reduce importance of charcoal rot by late summer irrigation.

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SCREENING OF POTATO FUNGICIDES IN 1957[1]

L. C. Callbeck[2]

Six fungicides for potato late blight (Phytophthora infestans (Mont.) de Bary) were compared in the Screening Plots of the Science Service Laboratory at Charlottetown, Prince Edward Island, in 1957. These fungicides were:

1. CR-1510 -- A confidential copper fungicide.
   Concentration: 4 pounds per 80 Imperial gallons.
   Source: Fisons Pest Control Limited, Cambridge, England, and received through International Fertilizers Limited, Quebec, Quebec.

2. Experimental fungicide -- A 2-to-1 mixture of maneb and tri-basic copper sulfate and containing a small portion of zinc sulfate.
   Concentration: 1.75-80.
   Source: Charlottetown Science Service Laboratory.

3. Dithane M-22 -- Maneb.
   Concentration: 1.5-80.
   Source: Rohm and Haas Company.

4. Dyrene 50-W -- 2,4-dichloro-6-(chloroanilino)-s-triazine.
   Concentration: 1.5-80.
   Source: Chemagro Corporation and received through Green Cross Products, Montreal, Quebec.

5. Mercurized Copper Oxychloride -- 50% copper, 0.6% mercury as phenyl mercury chloride.
   Concentration: 3-80.
   Source: F. W. Berk and Company Limited, London, England, and received through Green Cross Products.

6. Thioneb 50-W -- Polyethylene thiuram sulfides.
   Concentration: 1.5-80.
   Source: Naugatuck Chemicals, Elmira, Ontario.

In addition to the above six materials, Bordeaux mixture, 8-4-80, was added as a standard treatment, and an unsprayed control was included.

The plots were planted by hand on June 3, and the seed pieces were counted to assure the same number in each row. The variety Green Mountain was used. Each plot was 4 rows wide by 50 feet long; and eight plots, one for each treatment, were planted in each of four ranges. A row of potatoes was planted on each side of every plot to buffer the treatments; and, because these rows were not sprayed, it was believed that they served as sources of late blight infection. All data were taken from the two center rows of the plots.

To improve the possibilities of an epidemic a water suspension of late blight spores was sprinkled over the buffer rows on the evening of August 7; on the 16th of the same month, all

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Table 1. Percentage defoliation caused by late blight.

Treatment	Sept. 12	Sept. 18	Sept. 22
Bordeaux	0.5	6	18
CR-1510	8.0	44	72
Charlottetown Mixture	1.5	11	36
Dithane M-22	1.0	8	19
Dyrene 50-W	21.0	59	90
Mercurized Copper	0.5	6	18
Thioneb 50-W	12.0	52	92
Check	70.0	100	100

Table 2. Effect of treatments on yield and on late blight tuber rot.

Treatment	Total Bu/A	Smalls Bu/A	Rot Bu/A	No. 1 Bu/A	Percent rot
Bordeaux	431.7	43.6	5.7	382.4	1.3
CR-1510	440.6	46.6	18.5	375.5	4.2
Charlottetown Mixture	463.0	43.1	5.9	414.0	1.3
Dithane M-22	461.9	40.0	13.4	408.5	2.9
Dyrene 50-W	435.1	37.8	60.7	336.6	14.0
Mercurized Copper	408.6	41.6	2.9	364.1	0.7
Thioneb 50-W	437.6	40.0	26.0	371.6	5.9
Check	364.8	32.6	100.8	231.4	27.6
S. D. 5%	35.38			45.86	
S. D. 1%	48.16			62.42	

plots were given this treatment. The weather was very favorable for blight and the disease developed rapidly.

The sprays were applied with an experimental tractor-sprayer unit. The nozzles were so placed on the boom that each potato row received a 4-nozzle spray, and the fungicides were delivered at a pressure of 375 pounds per square inch. All rows were sprayed with DDT on July 3 and July 15, and with DDT and Malathion on August 13. The fungicides were applied July 17, 26, August 6, 16, 28, September 6, 13.

A few lesions were found in the buffer rows on August 12, and in the unsprayed control plots on August 19. On September 6, traces of blight were found in the plots for Bordeaux, CR-1510, Dithane M-22, and Mercurized Copper Oxychloride; Thioneb showed 1.0 percent defoliation, Dyrene 6.0 percent, and the controls 36 percent. The disease advanced rapidly, and in the control plots 90 percent of the foliage was destroyed by September 16. At that time it became apparent that Thioneb and Dyrene were not holding the disease, because in the plots sprayed with these fungicides 35 and 39 percent, respectively, of the foliage was destroyed.

Defoliation readings were taken at regular intervals from September 6 to September 22, and average defoliations for the later readings are presented in Table 1.

On September 24, or 113 days after planting, sodium arsenite solution was applied to destroy the remaining foliage. The plots were harvested on October 7, and the tubers were weighed, graded, and examined for late blight rot. The results are given in Table 2.

SCIENCE SERVICE, CANADA DEPARTMENT OF AGRICULTURE

THE EFFECT OF AIR MOISTURE ON THE PREDISPOSITION OF TOMATO TO BACTERIAL SPOT[1]

D. Davis and S. Halmos

Numerous isolates of Xanthomonas vesicatoria (Doidge) Dows. have failed to produce severe, consistent, or uniform infection of tomato foliage in our laboratory. Initial experiments were based on the work of Gardner and Kendrick (1) who demonstrated that tomato was susceptible to infection in the range of 59° to 90° F and that susceptibility increased the longer the plants were incubated in the moist chamber after inoculation. The work of various investigators (2, 3, 4) suggested that predisposition to bacterial diseases could be increased by subjecting the host tissue to high humidities prior to inoculation. Accordingly, an experiment was designed in which tomato plants were held in a moist chamber for various lengths of time prior to inoculation. The post-inoculation incubation period in the moist chamber was limited to a single time interval.

Greenhouse-grown tomato plants (Lycopersicon esculentum variety Bonny Best), with the sixth leaf just emerging, were inoculated with a bacterial suspension of X. vesicatoria. Strain B_2 of X. vesicatoria (obtained from G. Sowell, Jr.) was grown on PDA for 48 hours at 30° C. The bacteria were then washed from the surface of the PDA and suspended in distilled water (5). The bacterial suspension was diluted to yield a turbidity reading equivalent to a McFarland Standard No. 3 and sprayed only on the lower leaf surfaces with a No. 152 De Vilbis atomizer at 20 pounds pressure. Each plant received a 6-second burst of the bacterial suspension. All plants were inoculated at the same time. Since one group of plants was placed in the moist chamber prior to inoculation, their foliage was laden with water droplets at the time of inoculation. To equalize the inoculum density on all the plants, the group that was not incubated in the moist chamber before inoculation was sprayed with water just prior to inoculation. During the moist chamber incubation period the plants were maintained at 100 percent relative humidity and 30° C. Light was supplied daily for 12 hours from 40-watt "cool white" fluorescent tubes placed 18 inches above the tops of the plants. After the plants were removed from the moist chamber, the same light and temperature conditions were maintained for 6 days. A local lesion count was made 7 days after inoculation. Leaf area was obtained by determining the water displacement volume of a representative leaf for each leaf position. The water displacement volume was then related to the total leaf area of the representative leaves. The factor derived from this ratio was used to estimate individual leaf areas. Five replicates were used for each moist chamber treatment schedule. The experiment was repeated twice.

Table 1. The effect of high humidity on the predisposition of tomato to infection by Xanthomonas vesicatoria.

Number days plants at 100% relative humidity		Average number lesions per square centimeter Leaf position[a]							Average number lesions per plant
Prior to inoculation	After inoculation	1	2	3	4	5	6	Average	Total
0	0	0	0	0	0.01	0.05	0	0.02	4
0	1	0	0	0	0.04	0.30	0.27	0.15	27
1	1	0	0	0.49	1.31	3.34	1.04	1.45	354
2	1	0	0	8.07	4.87	5.54	1.25	4.63	1100

[a]Leaf 1 is the basal leaf. The other leaf positions follow in chronological order of their development.

[1]We wish to thank Arthur G. Itkin for the statistical analysis.

The following conclusions can be drawn from a statistical analysis[2] of the data, which are summarized in Table 1: 1) All moist chamber treatment schedules are significantly different from each other. 2) Within the limits of the experiment, the longer the period of time that tomato plants are exposed to 100 percent relative humidity prior to inoculation the greater their susceptibility to disease. 3) The two oldest leaves do not develop lesions, regardless of the moist chamber treatment schedule. 4) Where a moderate intensity of infection is obtained (moist chamber treatment 1 day prior to and 1 day after inoculation) there is an inverse correlation between leaf age and disease susceptibility for leaves 1 to 5; the younger the leaves, the greater their susceptibility to disease. The last conclusion does not hold for leaf 6, probably because this leaf was just emerging and many of its leaflets were only slightly expanded at the time of inoculation. The 2-day pre-inoculation moist chamber treatment schedule apparently failed to follow the leaf position pattern of susceptibility exhibited by the other treatment schedules. This may be due to the fact that disease incidence for this particular schedule was based on gross estimates of the number of local lesions, rather than on precise counts.

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[2]Statistical significance tested at P=.05.

STUDIES ON SWEETPOTATO STEM ROT OR WILT AND ITS CAUSAL AGENT

E. M. Hildebrand, C. E. Steinbauer, Charles Drechsler, and E. C. Tatman[1, 2]

Summary

This investigation sheds light on the cultural identity and pathogenic behavior of the sweetpotato Fusarium (F. oxysporum Schlecht. f. batatatis (Wr.) Snyder & Hansen), the causal agent of stem rot or wilt disease. A total of 36 cultural units or combinations thereof were tested 3 years in succession on three varieties -- very resistant Tinian, intermediate or moderately susceptible Triumph, and very susceptible Porto Rico. Of diverse origin, the five parent cultures were otherwise unidentified. For 3 years in succession the virulent parent and single-spore progeny strains of the fungus behaved essentially alike in degree and range of infection.

In the sporulation on sweetpotato stems macrospores predominated. Only microspores were produced by this Fusarium when it was grown in pure culture on potato-dextrose agar.

For use in the present breeding program, culture transfers at 3-month intervals, incubated at temperatures near 11° C, effectively maintained suitable pathogenic strains of the sweetpotato Fusarium.

Single-spore progeny cultures were obtained by means of the Modified Barber's Method. Of the five viable microspores isolated from the separate parent cultures the numbers that grew and gave rise to progeny cultures were: I, 5; II, 4; III, 5; IV, 5; and V, 4. Only one (I-6) of the macrospores isolated into culture was used. Thus 24 single-spore progenies were employed.

Three experiments, alike in all important particulars, were started soon after June 1 each year (1955, 1956, 1957) and ended, respectively, 21, 21, and 17 days later. Each experiment employed 108 10-inch clay pots containing a sterilized mixture of 2/3 well-composited clay loam and 1/3 clean sand. Each pot was planted with ten inoculated cuttings of one variety.

When the experiments terminated each plant was dug, examined, classified according to extent of injury, and given an arbitrary numerical "disease index" value based on five classes of injury.

The 1955 results showed a spread or range covering more than two classes, which was also true in 1956 and 1957, but to a lesser extent.

The higher temperatures in 1956 and 1957 than in 1955 apparently caused increases in severity of injury by the pathogen.

In 1955 four parents (I, II, III, IV) and their progenies behaved alike pathogenically. The means of the progenies were here consistently higher than those of the parents, though not significantly so. On the other hand the means for the progeny of Strain V were all significantly below the means of parent.

In 1956 the performance of the Fusarium parent and progeny cultures was again very similar for cultures I to IV, whereas the progeny of V made a remarkable recovery of virulence. This was

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[2] The authors are indebted for statistical analyses to E. James Koch, Biometrical Services, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

attributed possibly to the handling of the stock cultures which had been transferred at 3-month intervals between experiments.

The results of 1957 confirmed those of 1956. An apparent decline in virulence of culture IV and its progeny was not significant.

As expected, there were significant differences in the mean wilt indices among varieties. Among years, however, the average index values were, respectively, 62, 68, and 66, not significantly different. The higher index values for Triumph in 1956 and 1957 were found to be due to contamination or incipient infections in the cutting material.

In 1957, the shortening of the incubation period from 21 to 17 days caused a marked reduction in the average rating of Porto Rico as expected. For the successive increases in the average ratings for Tinian over the 3 years, the probable explanation is the increased temperatures of the test environment.

For Tinian, the mean indices for filtered originals, unfiltered originals, and the combined means of the single-spore progenies were, respectively, 17.6, 17.0, and 19.0; for Porto Rico 92, 95, and 91; for the three varieties combined, 65, 67, and 65; when the analyses combined the data from Tinian and Porto Rico (and omitted Triumph) the means were 55, 56, and 55. None of the differences within varieties or combined varietal comparisons were significant. Filtering appears unnecessary in the procedure for evaluating stem-rot or wilt resistance in sweetpotatoes, but does reduce frothing in the preparation of inocula.

INTRODUCTION

Stem rot or wilt is still one of the most important sweetpotato diseases, and breeding for resistance to it is an important essential in crop improvement. This disease appears to be indigenous to the United States. It was originally described by Halsted (1) in 1890 as due to Nectria ipomoea. Many years later, Harter and Field (2) reported finding a Fusarium to be the true causal agent. Shortly thereafter they (3) designated as causal agents two species of Fusarium (F. batatatis Wr. and F. hyperoxysporum Wr.) described by Wollenweber (11). Complete accounts of these appear in the monograph by Harter and Weimer (4).

In reporting, in 1940, on their species concept in the genus Fusarium, Snyder and Hansen (8) suggested a single species designation for the causal agent of the sweetpotato disease; namely, Fusarium oxysporum Schlecht. f. batatas (Wr.) Snyder & Hansen. Their endeavors were noteworthy in supplying simplicity for a most complicated genus of fungi, though presumably the term batatas should here be replaced by Wollenweber's genitive batatatis.

The primary objective of this investigation, which extended over 3 years, was to become familiar with the sweetpotato Fusarium through studies on species identity, cultural purification, and the conduct of operational procedures to maintain suitable pathogenetic strains for use in the present breeding program for disease resistance.

PRELIMINARY AND BASIC OBSERVATIONS

Sporulation

A study of the incidence of the sweetpotato stem rot, or wilt, was started in the autumn of 1954 in both the greenhouse and the field at Beltsville. Fruiting specimens were collected and examined for sporulation. Macrospores predominated on the stem-rot specimens whereas only microspores were produced when the fungus isolated into pure culture. Regardless of source, the macrospores were so much alike in their promiscuously variable main dimensions as to indicate a single Fusarium species.

Stock Cultures

Five old U. S. D. A. stock cultures of the sweetpotato Fusarium were examined. These were the residue from an original collection of about 40 that had been isolated and continu-

ously held in stock culture for a decade or longer, being used year after year for determining disease resistance. These cultures had been maintained on potato-dextrose or corn-meal agar for 6 months or longer between transfers. Once each year their pathogenicity had been tested on susceptible Porto Rico plants in contaminated soil in the greenhouse.

Test Procedure

The established test procedure for determining wilt susceptibility in sweetpotatoes had regularly employed a filtered composite of the five virulent stock cultures. The cultures were labeled: F-137, 7287c, 7313a, 7339b, and B-5941. They are cited herein as I, II, III, IV, and V, respectively. To eliminate bacterial contamination in stock cultures in the past, transfers had been made to Harter's Synthetic Agar[3] which supports good mycelial growth but suppresses bacterial growth.

When first examined, early in 1955, for condition of growth, one of the five old stock cultures was slightly lower in virulence and another was contaminated with bacteria. The contaminated culture was readily purified by a simple, rapid micrurgical technique (5).

Single-Cell Isolation

The pure culture concept (5), "the origin of a culture from a single cell, spore or unit, and from one only," is generally regarded as the prime essential for studying variability in microorganisms.

To prepare the stock cultures for the isolation of single spores, they were transplanted to potato-dextrose agar three times successively at weekly intervals. In single-spore isolation microspores were employed mainly, rather than macrospores, because of their simpler genetic constitution. Only microspores were produced in culture. Previously Wellman (10) had done single-spore culture work on the tomato Fusarium and presumably had used microspores, although he did not specify the spore type.

Single-spore isolations were made by the Modified Barber's Method (5) using the micromanipulative apparatus. By single cell isolation it is possible to purify most mass cultures which are usually mixtures of strains or units. To establish the stability of the five parent cultures (isolates or strains), single-spore progeny cultures were obtained from each of them. The numbers of single microspores which grew into culture were: I, 5; II, 4; III, 5; IV, 5; and V, 4. Only one macrospore was isolated into culture; namely, I-6. By the micrurgy used it was possible to follow every stage in the development of the several spores into cultures. The mycelium produced in culture by the lone macrospore was indistinguishable from the mycelia obtained from the microspores. Two spores proved to be non-viable when isolated into microdrops and failed to develop the swelling characteristic of the viable ones. To test the germinability requirements preliminary isolations were made in distilled water. The microspores failed to germinate there, but when potato-dextrose broth was added they germinated copiously.

When isolated into microdrops of liquid media (potato-dextrose broth), the microspores germinated within 8 hours and produced enough mycelial growth and spores for the mass to be visible to the naked eye within 24 hours. As the isolated spores were mounted on the underside of a coverslip which then was placed over a deep-well slide, thereby providing a moist chamber and incubator, it was convenient to observe their germination and growth from time to time. Sterile vaseline or petroleum jelly sealed the coverslip in place. At the bottom of the well a film of sterile culture medium was installed to maintain a uniform vapor tension so as to stabilize the culture droplets at the top. When the growth became visible to the naked eye, it was transferred to test-tube culture.

Identity of the Several Isolations

The isolations from sweetpotato stem rot or wilt that were employed in this study are all held referable to one species, Fusarium oxysporum Schlecht. f. batatatis (Wr.) Snyder & Hansen. During 1955, 1956, and 1957 examination was made of the varied reproductive bodies formed on the basal portions of the stems of the potted sweetpotato plants that had been inoculated with the five parent and 24 single-spore progeny cultures. In addition to numerous

[3] Harter's Synthetic Agar: Dextrose, 200 gm.; peptone, 10 gm.; NH_4NO_3, 10 gm.; KNO_3, 5 gm.; K_2HPO_4, 2.5 gm.; $MgSO_4$, 2.5 gm.; $CaCl_2$, 0.1 gm.; shredded agar, 20 gm.; distilled water, 1000 ml. pH 5.1. Sterilized at 110°C for 15 min.

microconidia closely resembling those produced copiously in agar cultures, scrapings from a diseased stem usually showed an abundance of macroconidia. These macroconidia in some instances appeared relatively uniform in dimensions, shape and septation; but much more often they showed the rather pronounced morphological variability that has long been familiar to investigators dealing with Fusarium. Marked variations were noted even when scrapings were taken from portions of stem only a few square millimeters in area -- from sporulating tracts presumably too small to have provided wide differences in environmental conditions.

The variations observed in the several isolations obtained originally from separate sources seem rather generally of haphazard occurrence. In Figure 1 are shown five groups of five spores (I-V:0) selected in 1956 as being approximately representative of the macroconidia produced on sweetpotato stems infected with the five separate parent isolations employed (I-V). Under each of these groups are shown three groups of five spores (I-V:1-3) selected the same year as being similarly representative of the macroconidia formed on sweetpotato stems infected with three single-spore subcultures isolated from each parent culture. Among the 20 groups of spores some groups differ rather markedly from most of the other 19 groups in the whole assortment, but the deviation shown in any group is not well sustained in the three other groups placed in the same column, which represent intimately related isolations. Variations in the macroconidia taken from diseased stems in 1955 and 1957 were measured and compared and appeared mainly of like fortuitous character. As no decisive characters could be recognized as distinguishing any one series of parent and progeny isolations from the other four series of cultures, all five series of cultures used are considered to belong to a single species.

MATERIALS AND METHODS

Three varieties of sweetpotatoes were selected: very resistant Tinian (P. I. 153655), very susceptible Porto Rico, and intermediate or moderately susceptible Triumph.

Ten days to 2 weeks before an experiment was started, cuttings were placed in sand for rooting. In 1955 and 1956, three- to five-node cuttings made from greenhouse-grown plants were used. In 1957 terminal-growth cuttings were made from sprouts produced in outdoor propagating beds. When ready they were pulled from the rooting bench and rinsed in tap water to remove sand, the basal ends were freshly cut with a sharp knife to remove the protective callus, and the roots were trimmed to 2 inches to facilitate the planting operation and provide additional openings for entrance of the pathogen. This severe pruning was more drastic than in ordinary commercial practice and favored the fungus over the host.

Five days before the experiment was begun, cultures were started in 150 ml. of nutrient solution[4] in 250 ml. Erlenmeyer flasks. After 5 days the following inocula were ready for use: 5 parent cultures singly, 24 single-spore progeny cultures singly, and a composite[5] of the 5 parent cultures, making a total of 30 sources. Added also were a series of 6 filtered cultures (5 single parents and 1 composite), making a grand total of 36.

The inoculum was prepared by diluting each 150 ml. cultural unit with distilled water to a volume of 400 ml. This was more than adequate in quantity and concentration. It was applied to one bundle of prepared rooted cuttings of each variety, making a total of 108 pot units for the 36 cultural units.

The experiments were started soon after June 1 in each of 3 years (1955, 1956, 1957). In each experiment 108 10-inch clay pots were employed, each containing a sterilized mixture of 2/3 well-composted clay loam and 1/3 clean sand. Each pot was planted with ten prepared cuttings of one variety, set in holes made by a pointed redwood dibble evenly spaced over the soil surface. Immediately before planting, the cuttings were dipped into the liter beaker containing 400 ml. of inoculum.

To prevent cross-infection (7) or cross-contamination great care was taken in all details of the inoculation procedure, with the three persons simultaneously performing different phases of the operations in different rooms.

One person, the plant preparator, removed the cuttings from the rooting bench, washed

[4] A modification of Steinbauer's liquid synthetic medium (1947): NH_4NO_3, 1 gm.; KH_2PO_4, 1 gm.; $MgSO_4$, 5 gm.; sucrose, 10 gm.; distilled water, 1000 ml.

[5] "Composite" preparation involves growing the five cultures separately and then mixing all the cultures, filtering off and discarding the liquid medium, macerating the filter paper and fungus growth in distilled water in a Waring Blendor and making the blend up to volume with distilled water for inoculation purposes.

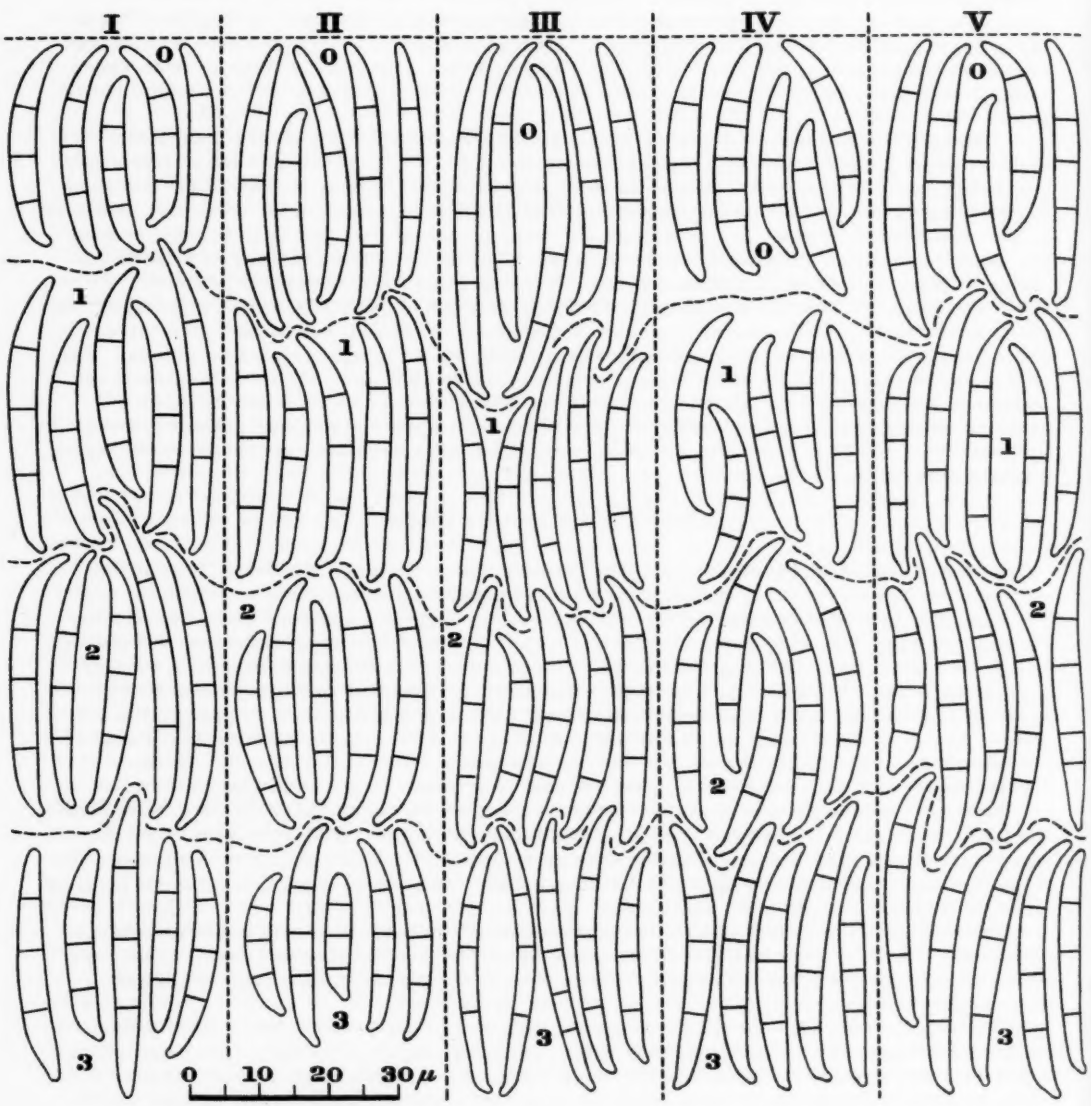

FIGURE 1. Groups of five macroconidia of Fusarium oxysporum f. batatatis produced on sweetpotato stems by the several parent isolations, I-V, and by three single-spore cultures obtained from each of the parent isolations, I-V: 1-3.

off adhering sand, trimmed roots and the basal stem callus; and delivered these freshly prepared healthy plants to the inoculation greenhouse. (In the 1956 and 1957 experiments, a fourth person carried the tray to the inoculation room.)

A second person, the culture preparator, assembled the several units of inoculum in the order of use according to the following procedure. The contents of each culture flask in most instances were emptied directly into a blending jar. In some fewer instances they were placed onto the filter in a Büchner funnel and after removal of the liquid by suction the filter paper together with the mycelial mat were placed in the blending jar. Then sterile distilled water was added to bring the volume to 400 ml. and the blender was operated for 30 seconds. The mycelial suspension was poured into a sterilized beaker and delivered to the greenhouse for use in inoculation. After each use the contaminated utensils were decontaminated, washed, rinsed successively in distilled water, 95% ethyl alcohol, and petroleum ether, and then drained dry before reuse.

A third person, the plant inoculator, used a sterilized dibble to make ten holes in the soil in each pot. The ten prepared cutting plants were dipped into the inoculum and then planted in the designated pots. The operator removed the contaminated beaker and dibble, thoroughly washed his hands, and dealt with the next lot of inoculum. This phase of the experiment required about 4 hours for completion.

After planting, the soil was watered and kept moderately moist during the test periods (21 days in 1955 and 1956, 17 days in 1957). Care was taken when watering to prevent cross-contamination by contact or splashing.

In the 1955 experiment, the test pots were arranged in numerical order with respect to the Roman and the Arabic numerals designating the isolations and subcultures used for inoculation, so that the three sweetpotato varieties were in juxtaposition on the benches. In the 1956 experiment, which duplicated the first one in most particulars, the sweetpotato varieties were placed on separate benches and the test pots or units within varieties were completely randomized. In 1957 the planting and pot arrangement were the same as in 1955.

At the termination of the experiment each plant was dug, examined with the aid of a sharp knife, classified according to the extent of injury from stem rot or wilt and given an arbitrary numerical value[6] based on severity of injury. The sum of the values for all plants in one pot gave an injury index number for that lot.

For confirming the identity of the causal agent in each of the 36 lots of diseased plants at digging time, several sporulating stem specimens were collected from the susceptible host series, placed on moistened filter paper in sterile Petri dishes and stored in the refrigerator (6° C).

Under these conditions the macrospores, which were produced in great abundance by all 36 cultural units during the third week of growth, seemed to survive very well. It was estimated that over 90 percent were alive at the end of 5 months' storage and that a considerable number, or over 30 percent, were still viable after 10 months' storage.

The severity of the disease on cuttings of the Triumph variety appeared to be greater in 1956 than in 1955. However, in 1957 some plants of this variety were found coming down with infection before any of the others. Thus the stock of this variety was found to be contaminated with incipient infections, giving higher values than expected in 1956 and 1957.

EXPERIMENTAL RESULTS

The experimental results are summarized in Table 1. This was essentially a comparative study that tested the established wilt-testing procedure and several alternatives. The several modifications took into account the identity and pathogenicity of the five parent cultures singly and in composite and 24 of their single-spore progeny, individually. The established procedure employed a filtered composite of five parent cultures, labeled VI-OF, which was compared with the effect of filtration on the five parent cultures, individually. The index number[6] represents the severity of disease induced by each of the 36 Fusarium culture units on the three sweetpotato varieties in 1955, 1956, and 1957.

The 1955 results, when examined for frequency distribution of inoculated plants among

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[6] The five classes of injury and their assigned numerical values for the 10-plant lots were: 1 -- dead, 10.0; 2 -- severe, 7.5; 3 -- moderate, 5.0; 4 -- slight, 2.5; and 5 -- none, 0.0. This is an adaptation of the commonly used indexing system which employs 25-plant samples in which the index values are, respectively, 4, 3, 2, 1, and 0. With either scale of values there is a maximum disease index of 100, if all plants are killed, and of 0 if no injury is evident.

Table 1. Summary compilation of the wilt index values for the experiments conducted during three successive seasons, representing the degree of severity induced by each of the 36 cultural units of Fusarium oxysporum f. batatatis on resistant Tinian, intermediate Triumph, and susceptible Porto Rico varieties of sweetpotato.

Varieties	Filtered Original				Unfiltered Original				Unfiltered Single-spore Progeny Cultures																							
									1				2				3				4				5				6				Progeny Mean			
Resistant Tinian	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean	'55	'56	'57	Mean
I	20	28	23	24	13	20	28	20	18	15	30	21	20	18	30	23	28	30	20	26	15	35	18	23	18	15	18	17	25	15	13	18	21	21	21	21
II	18	15	23	19	13	18	35	22	25	20	15	20	18	25	10	18	18	23	33	24	28	38	30	32									22	26	22	23
III	10	23	13	15	5	20	20	14	18	30	23	24	13	15	33	20	13	15	23	17	13	10	23	15	15	18	33	22					14	18	27	20
IV	20	18	10	16	5	8	17	10	20	8	25	18	15	8	25	16	8	15	13	12	13	13	10	12	8	15	5	9					13	12	16	13
V	13	20	10	14	10	20	23	18	5	33	23	20	10	18	20	16	10	23	8	14	15	15	30	20									10	22	20	18
VI (I-V)	10	25	30	22	5	20	38	21																												
Intermediate Triumph																																				
I	88	96	95	93	90	95	93	93	95	89	90	91	95	83	88	89	90	91	80	87	98	91	90	93	93	97	83	91	93	85	90	89	94	89	87	90
II	85	90	90	88	88	91	88	89	90	88	75	84	85	88	73	82	90	83	100	91	100	96	100	99									91	89	87	89
III	68	85	88	80	80	86	95	87	75	95	95	88	70	85	95	83	73	83	95	84	88	83	88	86	88	83	78	83					79	86	90	85
IV	53	89	85	76	78	83	75	79	88	80	83	84	75	79	85	80	73	83	88	81	55	71	70	65	80	90	78	83					74	81	81	79
V	48	95	78	74	85	92	100	92	63	86	85	78	23	91	95	70	40	83	90	71	20	80	83	61									37	85	88	70
VI (I-V)	88	100	93	94	80	100	98	93																												
Susceptible Porto Rico																																				
I	100	100	95	98	100	100	88	96	100	90	88	93	100	95	90	95	100	100	80	93	100	100	93	98	100	100	95	98	100	100	85	95	100	97	89	95
II	98	100	90	96	100	100	93	98	98	100	85	94	100	98	85	94	100	100	98	99	100	100	100	100									100	100	92	97
III	98	100	85	94	100	98	95	98	100	100	88	96	95	93	98	95	93	98	90	94	100	98	93	97	100	95	90	95					98	97	92	95
IV	85	98	80	88	100	100	72	91	100	100	88	96	100	93	68	87	98	98	70	89	93	90	78	87	98	85	90	91					98	93	79	90
V	93	93	73	86	90	100	88	93	85	98	80	88	65	98	88	84	65	95	85	82	50	68	80	66									66	90	83	80
VI (I-V)	98	100	88	95	95	100	98	98																												
Variety Totals R-I-S																																				
I	208	224	213	215	203	215	209	209	213	194	208	205	215	196	208	206	218	221	180	206	213	226	201	213	211	212	196	206	218	200	188	202	215	208	197	207
II	201	205	203	203	201	209	216	209	213	208	175	199	203	211	168	194	205	206	231	214	228	234	230	231									213	215	201	206
III	176	208	186	190	185	204	210	203	193	225	206	208	178	193	226	199	179	196	208	194	201	191	204	199	203	196	201	200					191	201	209	200
IV	158	205	175	179	183	191	164	179	206	188	196	197	190	180	178	183	179	196	171	182	161	174	158	178	186	190	173	183					185	186	176	182
V	154	208	161	174	185	212	211	203	153	217	188	186	98	207	203	169	115	201	183	166	85	163	193	147									113	197	191	167
VI (I-V)	196	225	211	211	180	220	234	211																												

the five injury classes, showed a spread or range often covering more than two classes as was also true in 1956 and 1957, but to a less extent. Such variability was believed due to insufficient uniformity among the cuttings. By more precise selection for age and maturity (Steinbauer, 9), this variability was demonstrably reduced in the cuttings. By repeating the experiment 3 years in succession, it was possible to observe the effects of time and frequency of transfer on the pathogenic stability of identical stock cultures. The higher temperatures in 1956 and 1957 appeared to favor the pathogen over the host, either through increase in virulence of the pathogen or decrease in resistance of the sweetpotato host, since there was an increase in severity of injury.

In Table 1 the index values, which represent the degree of severity, are arranged in juxtaposition for ready comparison of the three experiments. As a further aid in making comparisons, a cumulative index or sum of the variety totals is shown at the bottom of the table.

Four of the parents (I, II, III, IV) and their progeny cultures, taken individually in 1955, behaved pathogenically in similar fashion. From the cumulative index values the parents and their respective progeny means were: I, 203 and 215; II, 201 and 213; III, 185 and 191; and IV, 183 and 185. While the progeny means were consistently higher than those of these parents, the differences are small and not significant. The V values were 185 and 113. The strain V progeny means were significantly below those of the parent.

In 1956 the performance of cultures I to IV and their progeny was essentially the same as in 1955; whereas culture V and its progeny had apparently made a remarkable increase in pathogenicity: I, 215 and 208; II, 209 and 215; III, 204 and 201; IV, 191 and 186; and V, 212 and 197. The increase in pathogenicity of the single-spore progeny of V can possibly be attributed to the handling of the stock cultures, which had been simply transferred four times or at 3-month intervals between the two experiments.

The results in 1957 were: I, 209 and 197; II, 216 and 201; III, 210 and 209; IV, 164 and 176; and V, 211 and 191. In this experiment there was an apparent decline in virulence of culture IV and its progeny. However, when the values for all 3 years were taken into account, (Table 1) the differences between the years were not significant.

As expected, there were significant differences between varieties in regard to the mean wilt indices (Table 2). The average index values for the 3 years were 62, 68, and 66, respectively. Higher temperatures during the 1956 and 1957 seasons may account for at least

Table 2. Averages of index numbers for stem rot or wilt of the three sweetpotato varieties and for the three years of this investigation.

Variety	1955	1956	1957	3 Years
Tinian	15	20	22	19
Triumph	77	88	88	84
Porto Rico	94	97	87	93
Average	62	68	66	65

a part of this increase. The high index values for Triumph in 1956 and 1957 were found to be due to presence of incipient infections in the cutting materials. For Tinian to have shown progressive increases in the average ratings over the 3-year period, we have no explanation other than increased temperature. However, shortening the incubation period during 1957 from 21 to 17 days caused a marked reduction in the average rating of only the highly susceptible Porto Rico, which was expected. No similar reduction was noted in the other two varieties.

In the analysis of Tinian data alone, the differences between mean indices for filtered originals and unfiltered originals and the combined means of the single-spore progenies were not statistically significant, the means being, respectively, 17.6, 17.0, and 19.0. Also there were no significant differences between culture means within the filtered or unfiltered originals, which suggests that any one of these five parent cultures alone or two or more in composite can be used satisfactorily as sources of test inoculum. However, among the prog-

enies of different origin, there were some significant differences among the means, with the progeny of IV averaging significantly lower than the progenies of I, II, and III. Within parent cultures there were no significant differences among their single-spore progenies. For Tinian there were very highly significant differences among the means for the 3 years.

In the analyses of the Porto Rico variety by itself, no significant differences occurred between the filtered and unfiltered originals, and the single-spore progenies, with the means being, respectively, 92, 95, and 91.

In the analyses of all three varieties combined, the differences among the filtered originals, the unfiltered originals, and the single-spore progenies were not significant. The three means were, respectively, 65, 67, and 65. When the analyses combined the data from resistant Tinian and very susceptible Porto Rico (and omitted Triumph) the means were, respectively, 55, 56, and 55. Thus again the differences were not significant between the filtered and the unfiltered cultures.

Finally, filtration did not materially alter the extent of wilt injury, judging from the performance of the several Fusarium cultures on the three sweetpotato varieties in three experiments conducted during 1955, 1956, and 1957. Therefore, this step appears unnecessary and it can be discontinued in the procedure for evaluating stem rot or wilt resistance in sweetpotatoes. However, filtration is a convenience in preparing inocula since it eliminates frothing during maceration in the Waring Blendor.

DISCUSSION AND CONCLUSIONS

How many Fusarium cultures are necessary to guarantee successful evaluation of test materials for disease susceptibility or resistance? For the tomato, Porte and Wellman (6) found one stable culture of Fusarium oxysporum f. lycopersici (Sacc.) Snyder & Hansen satisfactory for determining wilt resistance in greenhouse tests. In this study it appears that any one of the five parent cultures used, or its progeny in a high state of virulence, gave dependable information on the relative resistance or susceptibility of the sweetpotato varieties to stem rot or wilt. Also, filtering was shown to have no significant effect on results. Thus the standard procedure can be simplified by omitting the filtration step. The stock cultures should be transferred quarterly or at 3-month intervals for maintaining the cultures in a high state of virulence. The possibility of occurrence of significant variation within a single culture must be kept in mind although the probability may be low.

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THE ABSORPTION OF STREPTOMYCIN BY BEAN PLANTS AS INFLUENCED BY GROWTH REGULATORS AND HUMECTANTS

R. N. Goodman and W. M. Dowler[1]

Summary

Two growth regulating substances, naphthyl acetamide and gibberellic acid, as well as a number of hydroxy-compounds increased the absorption of streptomycin by bean plants.

Conversely, both indole-3-acetic acid and a combination of methyl cellosolve and Carbowax 4000, when formulated with streptomycin, depressed the absorption of the antibiotic by the test plants.

Absorption of streptomycin was determined quantitatively by the cylinder-agar plate-diffusion method.

Numerous investigations have demonstrated the effectiveness of streptomycin in controlling plant diseases (22). These researches have provided both direct (13, 14) and indirect evidence (1, 5) that streptomycin acts systemically in plants as an antimicrobial agent. Furthermore, streptomycin has been found to act as both an eradicant (1, 5, 21) and as a protectant (3, 4, 5, 10, 16, 17, 20, 21).

Thus far the method of choice for applying antibiotics to plants in the field has been through foliar sprays. As a result, some workers have felt that the antibiotic applied in this fashion is only locally systemic or has but a limited systemic activity.

Rapid drying of the antibiotic-containing spray on the plant's surface and the waxy cuticle of the leaf are two obstacles which could conceivably limit absorption and penetration of the antibiotic. The problem of attaining the necessary inhibitory level of the antibiotic in plant tissue is further accentuated, by dilution of that which has been absorbed, through growth and translocation.

Thus, a number of studies have been directed at developing formulations which would permit more of the antibiotic that is applied to enter the plant.

Initially, attempts were made to reduce the rate of drying of the spray with lanolin emulsions and Carbowax 4000, a polyethylene glycol (5). Methyl cellosolve, an organic solvent, was employed to reduce the barrier presented by the cuticle (5). Some of these additions were effective in improving the disease-controlling properties of streptomycin *in vivo*. Later glycerol and other humectant-like substances were employed to delay drying of the antibiotic spray (7, 8). These substances were found to increase absorption of streptomycin by plants as well as to improve the *in vivo* activity of the antibiotic. Additional investigations showed indole-3-acetic acid, naphthyl acetamide and other growth regulators to increase suppression of plant pathogens *in vivo* by streptomycin (6, 10).

The work to be presented herewith was conducted to determine whether certain growth regulators as well as humectant-like compounds improved streptomycin absorption by plants.

METHODS

Antibiotic solutions containing humectants and plant growth regulators were applied as foliar sprays to bean plants (*Phaseolus vulgaris*), variety Tendergreen. The plants were sprayed when the first true leaves were near full size and the first trifoliate leaves were beginning to expand.

Eight plants were used in each treatment. The first two true leaves from each plant were harvested for bioassay 24 hours after the spray was applied. The 16 leaf-samples were washed for 20 minutes in running water, blotted dry, frozen, then thawed and the juices expressed in a Carver press at 2000 psi.

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The bioassay procedure employed was the cylinder-agar plate method described by Grove and Randall (9) with two modifications: (a) an 18-hour culture of Bacillus subtilis (ATCC-6633) diluted 1 to 100 was used instead of a spore suspension of this test organism, (b) the seeded plates were refrigerated for 4 hours prior to setting and filling the cylinders.

A standard curve was prepared each time that bioassays were performed. Points on the curve were 0.3, 0.5, 0.75, 1.0, 2.0, 4.0 and 5.0 mcg/ml with 2.0 mcg/ml being the reference or correction point for the curve.

A total of 18 plates was used in the preparation of the standard curve; three plates for each point on the curve with the exception of the 2.0 mcg/ml reference. Five cylinders were set in each plate, two of which were filled with the 2.0 mcg/ml reference and the remaining three with solutions of standard curve concentrations. Thus, each point on the curve represented an average of nine zones, whereas the average of 36 zones provided the reference point. Zones of inhibition were measured with a Fisher-Lilly antibiotic zonereader which is calibrated in tenths of millimeters.

Bean juice from each treatment was assayed in triplicate plates, with three cylinders in each plate containing the bean expressate and the remaining two the reference solution. The quantity of streptomycin detected in each treatment was derived from the averaged value of nine zones. This figure was corrected by the deviation of the averaged value of the six reference point-zones, in the plates containing bean juice, from the value of the reference point on the standard curve.

Bean juice diluted 1-5 in pH 8 buffer usually fell well within the 0.3-5.0 mcg/ml range of the assay.

All antibiotic, growth regulator and humectant concentrations were calculated on an activity basis.

RESULTS

The influence of hydroxy compounds on the absorption of streptomycin by bean foliage is presented in Table 1. From these data it is readily apparent that by adding glycerol, diethylene glycol (DEG), or triethylene glycol (TEG) to the streptomycin spray, the amount of antibiotic detected through bioassay of bean foliage was more than doubled. Conversely, the combination of methyl cellosolve (MC) and Carbowax 4000 (Carb.) resulted in a 22 percent decrease in assayable streptomycin.

The effect of the growth regulators, indole-3-acetic acid (IAA), naphthyl acetamide (NA), and gibberellic acid (GA), may be noted in Table 2. The inclusion of either NA or GA in the streptomycin spray formulation resulted in an increase in assayable streptomycin in bean juice of the same magnitude as was obtained with glycerol, DEG and TEG. Where IAA was added to the streptomycin spray, less antibiotic was detected in the bean expressate than where streptomycin alone was applied.

Table 3 discloses the effect of indole-3-acetic acid in streptomycin absorption when formulated with streptomycin alone, MC-Carb. combinations and with glycerin. In all three treatments, IAA depressed streptomycin absorption. Thirty-four percent less antibiotic was detected in foliage receiving IAA plus streptomycin than where streptomycin alone was applied. This antagonistic effect of IAA is even more apparent when one compares the glycerin-streptomycin treatment with the one which combines these two compounds with IAA. In this instance the reduction in assayable streptomycin is 7.15 mcg/ml or a decrease of 63 percent. As compared with streptomycin alone, the treatment including IAA, MC, and Carb. in the formulation resulted in a decrease of 30 percent in absorption of the antibiotic.

DISCUSSION

To many who have been working with antibiotics to control plant disease it is apparent that these compounds are responsible for the most significant advance made in plant chemotherapy in recent years. The excellent results obtained with streptomycin in combatting such diseases as fireblight of apple and pear, black spot of tomato and pepper, walnut blight, and others, are testimonies of this.

A review of the literature pertaining to the control of plant bacterial pathogens immediately reveals the fact that streptomycin is overwhelmingly the antibiotic of choice for this purpose. Therefore, until a superior antimicrobial agent is discovered, a problem of major concern to phytopathologist would appear to be the development of methods whereby the efficacy of streptomycin might be increased.

Table 1. Effect of hydroxy-compounds on the absorption of streptomycin (200 mcg/ml) by bean foliage.

Additive*	Replicates per treatment	mcg/ml Antibiotic detected (average of replicates)
None	8	4.73
Glycerol 1%	7	11.21
TEG 1%	4	11.76
DEG 1%	2	13.02
MC 1% + Carb. 1%	1	3.69

*TEG = triethylene glycol; DEG = diethylene glycol; MC = methyl cellosolve (ethylene glycol monomethyl ether); Carb. = Carbowax 4000 (polyethylene glycol).

Table 2. Effect of growth regulators on the absorption of streptomycin (200 mcg/ml) by bean foliage.

Additive*	Replicates per treatment	mcg/ml Antibiotic detected (average of replicates)
None	8	4.73
NA 20 mcg/ml	6	11.20
GA 30 mcg/ml	2	11.58
IAA 100 mcg/ml	2	3.10

*NA = naphthyl acetamide; GA = gibberellic acid; IAA = indole-3-acetic acid.

Table 3. Effect of indole-3-acetic acid on the absorption of streptomycin (200 mcg/ml) by bean plants.

Additive*	Replicates per treatment	mcg/ml Antibiotic detected (average of replicates)
None	8	4.73
IAA	2	3.10
MC + Carb.	1	3.69
IAA + MC + Carb.	2	3.30
Glycerol	7	11.21
IAA + Glycerol	2	4.08

*Additives were used at the concentrations noted in Tables 1 and 2

It is both natural and logical that one of the most popular approaches to this problem should be the development of methods whereby the levels of streptomycin in plant tissues might be increased. For the past 3 years our studies with growth regulators and humectants have been directed specifically at this facet of the overall problem.

The results from our most recent experiments with hydroxy compounds are, qualitatively, in good agreement with the findings of Gray (7, 8). Furthermore, the results obtained with naphthylacetamide and gibberellic acid presented herein, and the improved disease control obtained with a number of other growth regulating substances when formulated with streptomycin (6, 10), suggest that these compound also may increase the absorption of streptomycin by plants.

Attention should be called, at this point, to the dissimilarities between these two groups of chemical compounds. The differences in chemical configuration are quite obvious. The humectants are essentially di- and tri-hydroxy-compounds, whereas a large number of growth regulators are derivatives of indole, benzene, naphthalene, fluorene (gibberellic acid), phenoxy, and naphthoxy compounds. Further, the activity of these hormone-like materials is frequently governed by organic acid side chains of variable length, and by the degree of halogenation.

Their influence, physiologically, upon plants seems also to be widely divergent. The hydroxy-compounds might be expected to increase the hydration of the cuticle and the cell walls and in this way influence the absorption of the water-soluble streptomycin molecules.

On the other hand, the literature abounds with information regarding such effects of growth regulators as increased rate of respiration, cell enlargement, and rate of water uptake. More specifically, Pramer (17, 18, 19) has recently demonstrated that in Nitella clavata the uptake of streptomycin requires metabolic energy. Thus, if the absorption of this antibiotic by higher plants is not a matter of passive diffusion, but if, instead, it is actively absorbed, then growth regulators might well influence this phenomenon.

The data obtained with IAA and with the methyl cellosolve-carbowax combination appear to be paradoxical, since both have increased the efficacy of streptomycin in controlling Erwinia amylovora in artificially inoculated apple shoots (6), yet our data strongly suggest that both materials depress the absorption of streptomycin.

Finally, it is very obvious that additional experimentation is sorely needed particularly in discerning the factors responsible for streptomycin absorption, per se and as modified by humectants and growth regulators.

In this respect, we are currently conducting experiments in which such factors as temperature, day length, light intensity, and relative humidity are being closely regulated.

Once some of the mechanisms involved are elucidated, it may be possible to predict with a reasonable amount of success additional groups of chemical compounds capable of increasing streptomycin absorption by plants.

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VALUE OF INSECTICIDE-FUNGICIDE COMBINATION TREATMENTS AS PROTECTANTS FOR SEED OF CUCUMBER AND WINTER SQUASH[1]

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Summary

Captan (Orthocide 75) and thiram (Arasan SF) each at two dosage rates, were combined with dieldrin, heptachlor, and lindane to provide 1:4 and 1:8 ratios of insecticide to fungicide at each dosage for treatment of cucumber and squash seed. Insecticide-fungicide combination treatments, regardless of dosage of the pesticides, gave better total stands, in most instances, than treatments with fungicide alone. In fine sandy loam, thiram and captan were equally effective on cucumber and squash seed. In silt loam, the fungicides were equally effective on cucumber seed, but captan was better than thiram on squash seed. On the basis of total emergence of cucumber seeds, dieldrin was better than heptachlor and lindane; no differences were observed among the insecticides on squash seed. Combination treatments effectively controlled seed-corn maggot injury on squash seed and resulted in a higher percentage of normal plants than treatments with fungicide alone.

An increasing number of growers in New York State are adopting the practice of treating cucurbit seed with an insecticide-fungicide combination treatment to prevent injury by the seed-corn maggot Hylemya cilicrura (Rond.) and seed rotting fungi. Although excellent stands following such practice have been reported, the value of these treatments has not been determined under experimental conditions in New York State. In California, Lange et al. (2) observed that benzene hexachloride combined with Arasan had no adverse effect on cucumber seed and that treatment of cantaloupe seed with benzene hexachloride or DDT, alone or in combination with Semesan, caused no reduction in emergence from seed planted in pasteurized soil. In soil infested with Pythium spp., Semesan alone and in combination with either benzene hexachloride or DDT gave excellent control of pre- and post-emergence damping-off. Other workers in California reported no adverse effects on germination of seed in field plantings of nine varieties of cucurbits treated with lindane or dieldrin when combined with thiram but indicated that in some instances the insecticides alone reduced emergence (1). In plantings where the seed-corn maggot was injurious, stands were considerably improved by the use of the combination treatments. Wallen, et al. (3) found that combination insecticide-fungicide seed treatments on all cucurbits tested improved emergence. Even though the seed-corn maggot was not injurious in their experimental plantings of these vegetables in any of three Canadian locations, they recommend inclusion of an insecticide in seed treatment for cucurbits.

Tests were conducted in 1956 to obtain data under New York conditions on the value of insecticide-fungicide combination treatments as protectants against soil-borne insect and fungal pests of the seed of cucumber and winter squash.

MATERIALS AND METHODS

All tests were conducted in the field. Seed of National Pickling cucumber and Blue Hubbard squash were used. The seeds were treated with laboratory preparations containing the fungicide captan (Orthocide 75) or thiram (Arasan SF) in combination with each of the insecticides dieldrin (75% w.p.), heptachlor (75% w.p.), or lindane (75% w.p.). The commercial seed treatment preparations Delsan A-D (thiram plus dieldrin), Ortho Seed Guard (captan plus lindane), and GLF Combination Seed Treatment # 1 (thiram plus lindane) were also included in the tests.

All of the seed treatment mixtures were applied to cucumber seed in slurry form. Dieldrin plus captan and dieldrin plus thiram were also applied as dusts. Slurries of the various

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laboratory preparations were prepared by suspending the required amount of fungicide and insecticide, or fungicide alone, in a measured volume of a 2% methocel solution. The fungicides were applied at dosage rates of 0.26% and 0.52% actual toxicant by weight of the seed. Each insecticide was applied at rates of actual toxicant sufficient to provide a 1:4 and a 1:8 ratio to each of the fungicides at the two dosage rates. The slurries were used at a volume of 1 1/8 pints per 28 pounds of seed. The ratio of insecticide to fungicide in the commercial preparations was not altered. These preparations were applied at the rate of 0.26% actual fungicide by weight of the seed.

The pesticides were applied to squash seed in the form of dusts, except for one slurry treatment with captan plus lindane, and slurry treatments of the commercial preparations. With dust treatments, each fungicide was used at rates of 0.13% and 0.26% of actual toxicant by weight of the seed. Each insecticide was applied at rates that provided ratios of 1:4 and of 1:8 of insecticide to fungicide. The commercial preparations were applied in slurry form at 0.13% actual fungicide by weight of the seed without any change of the ratio of insecticide to fungicide. The slurries were applied at the rate of 1 1/8 pints per 28 pounds of seed.

To treat the cucumber and squash seeds, the required amount of slurry or dust was added to a weighed lot of seed contained in a glass jar. The jar was then attached to a motor-driven wheel and rotated slowly for 3 minutes to obtain a uniform coverage of the pesticide preparation. All treated seeds were held at 65° to 75° F until planting time.

The seeds were planted by means of a Planet Jr. seeder from which the seeding plate had been removed to permit the ready flow of the treated seed through the opening at the lower end of the seeder box. As the seeder was pushed along the row at an even pace, the seeds were hand-fed into the seeder in such a manner that they were uniformly distributed along the row. Each plot consisted of a single row 10 to 12 feet in length and occurred in random order in each of six blocks.

In plantings of cucumber seed, 100 seeds were planted per row and in plantings of the squash seed, 50 seeds were planted per row. One planting of the cucumber and squash seed was made on May 22 in fine sandy loam and a second planting on May 24 in silt loam. Immediately after seeding, tankage was distributed in a band over the surface of each row to encourage the deposition of eggs by adults of the seed-corn maggot. Data on emergence and maggot injury from the first planting were obtained on June 4 and from the second planting on June 11.

RESULTS

Cucumber Seed Treatments: All treatments improved emergence in both fine sandy loam and in silt loam (Table 1). Treatments containing an insecticide were more effective, in most instances, than those lacking an insecticide. Dust treatments were comparable to slurry treatments. Results from treatments with fresh laboratory preparations of insecticide-fungicide combinations were similar to those from commercial preparations. Analysis of the factorial portion of the experiments indicated no differences between captan and thiram treatments nor between dosage rates. In fine sandy loam, emergence from treatments containing dieldrin was greater than that from treatments containing heptachlor or lindane (Table 2). The difference between heptachlor and lindane treatments was not significant. In silt loam, emergence from treatments containing dieldrin and those containing heptachlor was comparable and significantly greater than that from treatments containing lindane. In fine sandy loam, emergence from treatments in which the ratio of insecticide to fungicide was 1:4 was greater than from those in which the ratio was 1:8, regardless of fungicide dosage. Emergence from treatments containing dieldrin at a ratio of 1:8 did not differ from that from treatments in which the ratio was 1:4. Heptachlor and lindane, however, were less effective at the 1:8 ratio than at the 1:4. In silt loam, emergence from treatments at an insecticide-fungicide ratio of 1:8 was greater when the fungicide was used at the rate of 0.26% than at 0.52% by weight of seed. At a ratio of 1:4, the higher fungicide dosage was more effective.

Squash Seed Treatments: Plants from untreated seed and from treatments lacking an insecticide showed considerable injury by the seed-corn maggot. This was especially true of the planting in fine sandy loam where in treatments lacking an insecticide about 40 percent of the plants which emerged were injured (Table 3). The plumule on many of the maggot-injured plants was injured to such an extent that the growing point was killed. Such plants did not develop beyond the cotyledonary stage. Dieldrin and lindane were slightly more effective than heptachlor in preventing maggot injury. Results from dust and slurry treatments were similar. Analysis of the factorial portions of the experiments indicated that captan and thiram

were equally effective in fine sandy loam, but captan was more effective than thiram in silt loam (Table 4). In fine sandy loam, emergence at the fungicide rate of 0.13% by weight of seed was similar to that at 0.26%. In silt loam, emergence was slightly greater at the higher fungicide dosage. The differences in emergence between the insecticides were not significant. In silt loam, emergence from treatments with a 1:4 ratio of insecticide to fungicide was slightly greater than at a 1:8 ratio. In fine sandy loam, captan at 0.13% by weight of seed was more effective than at 0.26%; whereas, with thiram, the higher dosage was more effective. In silt loam, each of the fungicides was more effective at the higher dosage.

Table 1. Emergence of plants from National Pickling cucumber seed treated with fungicide alone and with combination treatments containing different ratios of insecticide to fungicide.

Insecticide	Fungicide: Material	Fungicide: % by wt. of seed [a]	Ratio of insecticide to fungicide [b]	Emergence [c]: Fine sandy loam per cent	Emergence [c]: Silt loam per cent
Dieldrin	Orthocide 75	0.03	1:8	64	40
"	"	0.06	1:8	70	44
"	"	0.06	1:4	67	48
"	"	0.12	1:4	66	38
"	Arasan SF	0.26	1:8	68	50
"	"	0.52	1:8	62	38
"	"	0.26	1:4	65	45
"	"	0.52	1:4	67	50
Heptachlor	Orthocide 75	0.26	1:8	61	46
"	"	0.52	1:8	58	41
"	"	0.26	1:4	65	42
"	"	0.52	1:4	63	45
"	Arasan SF	0.26	1:8	62	41
"	"	0.52	1:8	58	45
"	"	0.26	1:4	64	40
"	"	0.52	1:4	67	43
Lindane	Orthocide 75	0.26	1:8	58	36
"	"	0.52	1:8	55	34
"	"	0.26	1:4	64	41
"	"	0.52	1:4	62	38
"	Arasan SF	0.26	1:8	52	41
"	"	0.52	1:8	58	34
"	"	0.26	1:4	64	35
"	"	0.52	1:4	66	46
None	Orthocide 75	0.26	---	53	34
	"	0.52	---	52	41
	Arasan SF	0.26	---	50	25
	"	0.52	---	56	33
None	None	----	---	38	19
Dieldrin	Orthocide 75 [d]	0.26	1:4	66	34
"	Arasan SF [d]	0.26	1:4	68	42
Delsan A-D		0.26		64	48
Ortho Seed Guard		0.26		67	39
GLF Combination Seed Tr. #1		0.26		63	33
Least difference for significance at 5%				8	10
" " " " at 1%				11	13

a Based on active ingredient, pesticides applied in slurry form except where otherwise indicated.

b Ratio is based on amount of active ingredient of each pesticide.

c Average emergence from 6 replications of 100 seeds each.

d Treatments applied as dusts.

Table 2. Summary of treatment effects on emergence from cucumber seed treatment factorial experiment.[a]

MAIN FACTORS		Emergence: Fine sandy loam (Per cent)	Emergence: Silt loam (Per cent)
Fungicides			
Orthocide 75		63	41
Arasan SF		63	42
		ns	ns
Fungicide Rates			
0.26 per cent by weight of seed		63	42
0.52 per cent by weight of seed		63	41
		ns	ns
Insecticides			
Dieldrin		66	44
Heptachlor		62	43
Lindane		60	38
LSD (99:1)		4	5
Insecticide Ratios			
Ratio of insecticide to fungicide			
1:8		61	41
1:4		65	42
LSD (99:1)		3	ns
INTERACTIONS [b]			
Fungicide Rates x Insecticide-Fungicide Ratios			
Fungicide Rate	Insecticide Ratio		
0.52%	1:8	60	39
	1:4	65	43
0.26%	1:8	61	42
	1:4	65	41
		ns	**
Insecticides x Insecticide-Fungicide Ratios			
Insecticide	Ratio		
Dieldrin	1:8	66	43
	1:4	67	45
Heptachlor	1:8	60	43
	1:4	65	43
Lindane	1:8	56	36
	1:4	64	40
		**	ns

a ns = differences not statistically significant.
** = significant at odds of 99:1.

b The following interactions were not statistically significant: Fungicides x Fungicide Rates, Fungicides x Insecticides, Fungicides x Insecticide Ratios, and Fungicide Rates x Insecticides.

Table 3. Emergence of plants from Blue Hubbard squash seed treated with fungicide alone and with combination treatments containing different ratios of insecticide to fungicide.

Insecticide	Material	% by wt. of seed [a]	Ratio of insecticide to fungicide [b]	Fine sandy loam		Silt loam
				Emg.[c] Percent	Maggot-injured plants Percent [d]	Emg.[c] Percent
Dieldrin	Orthocide 75	0.13	1:8	88	0.0	80
"	"	0.26	1:8	84	2.4	80
"	"	0.13	1:4	88	0.0	80
"	"	0.26	1:4	86	0.0	86
"	Arasan SF	0.13	1:8	88	0.0	68
"	"	0.26	1:8	88	0.0	76
"	"	0.13	1:4	88	2.3	76
"	"	0.26	1:4	86	0.0	78
Heptachlor	Orthocide 75	0.13	1:8	88	0.0	84
"	"	0.26	1:8	82	0.0	84
"	"	0.13	1:4	88	2.3	82
"	"	0.26	1:4	88	0.0	86
"	Arasan SF	0.13	1:8	86	4.7	68
"	"	0.26	1:8	88	6.8	76
"	"	0.13	1:4	84	2.4	74
"	"	0.26	1:4	88	2.3	76
Lindane	Orthocide 75	0.13	1:8	84	4.8	80
"	"	0.26	1:8	84	2.4	80
"	"	0.13	1:4	88	0.0	86
"	"	0.26	1:4	82	0.0	86
"	Arasan SF	0.13	1:8	88	0.0	76
"	"	0.26	1:8	84	0.0	78
"	"	0.13	1:4	84	0.0	76
"	"	0.26	1:4	92	0.0	80
None	Orthocide 75	0.13	---	78	32.5	80
"	"	0.26	---	76	42.1	72
"	Arasan SF	0.13	---	78	41.0	66
"	"	0.26	---	76	39.5	72
"	None	----	---	58	55.2	52
Lindane	Orthocide 75 [e]	0.13	1:4	84	4.8	84
Delsan A-D [e]		0.13		84	0.0	56
Ortho Seed Guard [e]		0.13		86	0.0	82
GLF Comb. Treatment #1 [e]		0.13		82	2.4	70
Least difference for significance at 5%				8	---	8
	at 1%			12	---	12

a Dosage based on active ingredient; pesticides applied as dusts except where otherwise indicated.

b Ratio based on amount of active ingredient of each pesticide.

c Percentage emergence based on 6 replications of 50 seeds each.

d Based on number plants emerged.

e Treatments applied in slurry form.

Table 4. Summary of treatment effects on emergence from squash seed treatment factorial experiment.[a]

MAIN FACTORS	Emergence: Fine sandy loam, Per cent	Emergence: Silt loam, Per cent
Fungicides		
Arasan SF	87	76
Orthocide 75	86	83
LSD (99:1)	ns	3
Fungicide Rates		
0.13 per cent by weight of seed	87	77
0.26 per cent by weight of seed	86	81
LSD (99:1)	ns	3
Insecticides		
Dieldrin	87	78
Heptachlor	86	79
Lindane	86	80
	ns	ns
Insecticide Ratios		
Ratio of insecticide to fungicide		
1:8	86	78
1:4	87	80
LSD (19:1)	ns	2
INTERACTIONS[b]		
Fungicides x Fungicide Rates		
Arasan SF 0.13% by wt. of seed	86	73
Arasan SF 0.26% by wt. of seed	87	78
Orthocide 75 0.13% by wt. of seed	87	82
Orthocide 75 0.26% by wt. of seed	84	83
	**	ns

a ns = differences not statistically significant.
** = significant at odds of 99:1.

b The following interactions were not statistically significant: Fungicides x Insecticides, Fungicides x Insecticide Ratios, Fungicide Rates x Insecticides, Fungicide Rates x Insecticide Ratios, and Insecticides x Insecticide Ratios.

DISCUSSION

These experiments demonstrated that insecticide-fungicide combination treatments improved emergence of cucumber and squash seed under conditions favorable for activity of the seed-corn maggot and seed decay organisms. Seedlings from seed treated with combination treatments frequently were more vigorous than those from seed treated with fungicide alone or from untreated seed.

Dieldrin in combination with either captan or thiram was superior to heptachlor and lindane for the protection of cucumber seed. Frost et al. (1) had previously observed that dieldrin was superior to lindane for treatment of cucumber seed. Captan and thiram were equally effective as the fungicide component of the combination treatments for cucumber seed.

The ratio of insecticide to fungicide in combination treatments may be of importance to cucumber. Emergence from treatments in which the ratio of insecticide to fungicide was 1:4 was greater than that from treatments in which the ratio was 1:8.

These experiments indicated that captan was superior to thiram in combination treatments for protection of squash seed in a silt loam. In a fine sandy loam, thiram was equal to captan in effectiveness. In the latter soil, captan appeared to cause hardening of seed coats of winter squash. This condition was not serious, but it may have contributed to the lower emergence from seed treated with captan at 0.26% by weight of seed than at 0.13%.

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EFFECT OF SEED TREATMENTS ON DEVELOPMENT OF ANTHRACNOSE IN WATERMELON SEEDLINGS

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Summary

Semesan, Arasan, and Spergon applied as protectants to watermelon seed at recommended rates did not control anthracnose (Colletotrichum lagenarium) satisfactorily on artificially infested seed. Increasing the rate of fungicide applied, either as a dust or in methocel slurries, had no significant effect on emergence or disease control. Bichloride of mercury treatments gave excellent results in most cases but were inconsistent, in that anthracnose was not satisfactorily controlled in all tests and in one instance a significant delay in emergence occurred.

Seedox, Panogen, and Emmi gave almost complete anthracnose control and under natural conditions should perform well in an anthracnose control program, but additional testing on other varieties is desirable.

INTRODUCTION

Among materials which have been judged capable of eradicating anthracnose spores (Colletotrichum lagenarium (Pass.) Ell. and Halst.) from watermelon seed are Semesan (30% hydroxymercurichlorophenol), Spergon (96% chloranil), and Arasan (50% thiram) (5,6). In preliminary experiments in both greenhouse and field in South Carolina these chemicals and others, however, gave disappointing results in the control of seed-borne anthracnose. Similar findings have also been obtained by Rankin (7) in Georgia.

The present study was designed to determine the efficiency of various fungicidal preparations against seed-borne anthracnose spores, their effect on seedling emergence and survival when applied to non-infested seed, and also the influence of various fungicidal rates and methods of application on incidence of disease and injury.

MATERIALS AND METHODS

Seed of Florida Giant, a variety highly susceptible to anthracnose, was used throughout. All cracked and visibly defective seed were discarded. The entire quantity required for each experiment was thoroughly mixed before being divided into sub-lots.

Seed were infested with anthracnose spores by soaking 30 minutes in a suspension of conidia obtained from 7-day-old bean pod cultures. The concentration of the spore suspension varied somewhat in each experiment but an attempt was made to have about 30 spores per high-power field. After soaking, the seed were dried under a fan at room temperature to their original weight. In all experiments infested and non-infested lots were compared in each fungicide or method treatment.

Dust treatments were applied by adding the required amount of fungicide to the appropriate sub-lot of seed in fruit jars and shaking for 5 minutes. Sub-lots treated with bichloride of mercury were rinsed in running water after treatment and allowed to dry. After other liquid treatments the seed were allowed to dry without rinsing.

In slurry treatments the proper amount of fungicide was suspended in enough 3% Methocel (dimethyl ether of cellulose) to coat the seed evenly.

Both field and greenhouse studies were conducted, but, because of the ease of secondary spread in the greenhouse by watering, greater reliance was placed on data from field plantings. In field tests non-infested lots were planted in each plot adjacent to those sown with infested ones so that secondary spread could be easily detected. Only data from field experiments in

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which no secondary spread occurred are reported here.

In the field seed were planted about 8 inches apart at a depth of 1 1/2 inches in rows 4 feet apart. Uniform depth was secured by the use of a marking wheel. Usually 100 seed were planted per plot, and replicated from three to five times.

Diseased seedlings were collected periodically and brought into the laboratory for examination or culture. Presence of setae on the hypocotyl or cotyledon were regarded as proof that the anthracnose fungus was involved.

EXPERIMENTAL RESULTS

The poor control of seed-borne anthracnose in preliminary experiments suggested that the amount of fungicide applied was not sufficient to suppress development of the organism when large numbers of spores were present on the seed coat. Accordingly, infested and non-infested seed lots were treated with three different rates of each fungicide. Dry materials were applied at rates of 0.0625, 0.125 and 0.25 percent by weight. The highest rate approximated the maximum amount of dust that would adhere to the seed. Bichloride of mercury and Vancide 51 (30% sodium dimethyl dithiocarbamate and sodium derivative of 2-mercaptobenzothiozole) were used as 10-minute soaks at concentrations of 1:2000, 1:1000, and 1:500.

Statistical treatment of the stand and disease data indicated no differences due to rates of application (Table 1). The fungicide x rate interaction was not significant, although it was high enough to suggest that under certain conditions real differences between rates might occur. Bichloride of mercury at 1:500 concentration gave a consistently, although not significantly, lower stand than the two lower rates.

Performance of the various fungicides in controlling anthracnose varied. Best results based on the number of surviving healthy plants (Table 2) were obtained by the use of Vancide 51, Seedox (50%, 2,4,5-trichlorophenylacetate) and the two bichloride of mercury treatments. Semesan gave poorest control of all. A high negative correlation (-0.935) existed between mean stand of healthy plants and percentage of plants killed by the anthracnose fungus (Table 3).

Symptoms most frequently observed were drooping and wilting of the cotyledons, with sunken lesions on the hypocotyl near the ground line (Fig. 1). Cotyledonary lesions occurred rarely.

FIGURE 1. Watermelon seedlings showing anthracnose lesions near the ground level. Healthy plant at left.

Phygon (50% dichlone), Carbide and Carbon 5400 (75% alpha, alpha, trithiobis N-dimethyl thioformamide), Arasan, and Spergon resulted in significantly greater stands than any of the other materials where non-infested seed were used. The two bichloride treatments, on the other hand, significantly reduced stand below that of any other treatment and also caused delay in emergence when compared with several of the dust treatments (Table 1).

Table 1. The influence of seed treatment on final stand of healthy watermelon seedlings.

Fungicide	Mean number of plants a: Seed artificially infested with spores of *C. lagenarium*	Mean number of plants a: Non infested Seed	Mean for seed lots
Semesan	4.7	67.4	36.1
Arasan	17.0	73.3	45.2
Captan	18.2	65.7	42.0
Carbide and Carbon 5400	21.2	74.0	47.6
Phygon	23.0	77.6	50.3
Thiram (U.S. Rubber Co.)	26.5	69.2	47.8
Spergon	29.4	71.6	50.5
Dow F-1112 c	29.8	64.2	47.1
Bichloride of mercury followed by Arasan	42.6	52.6	47.6
Bichloride of mercury	44.1	49.2	46.6
Vancide 51 (soak)	50.6	63.3	57.0
Seedox	52.1	67.2	59.7
Vancide 51 (slurry)	56.0	68.5	62.3
Control b	15.2	63.3	39.8
LSD (.05)	7.6	7.6	5.5
LSD (.01)	10.1	10.1	7.3

[a] Means based on 3 rates of fungicide and 3 replications. Rate means (.05) were not significantly different. See text.
[b] Control not included in statistical analysis.
[c] Zinc trichlorophenate.

Table 2. The influence of seed treatment on number of plants infected with Colletotrichum lagenarium[a].

Fungicide	Mean percentage of plants infected by C. lagenarium. [b]	
	Angle [c]	Percent
Bichloride of mercury	9.9	3.0
Seedox	12.2	4.5
Vancide 51 (slurry)	13.9	5.8
Bichloride of mercury followed by Arasan	14.9	6.6
Vancide 51 (soak)	19.3	10.0
Dow F-1112	26.9	20.5
Spergon	29.5	24.3
Captan	32.7	29.2
Carbide and Carbon	33.9	31.1
Arasan	35.0	32.9
Thiram (U.S. Rubber)	37.4	36.9
Phygon	38.1	38.1
Semesan	50.7	59.9
Control	39.0	38.6
LSD (.05)	14.9	-
LSD (.01)	20.2	-

[a]Artificially infested seed were used.

[b]Means based on three rates of fungicide and three replications. Rate means were not significantly (.05) different. See text.

[c]Angle = arc sin $\sqrt{\text{percentage}}$.

SLURRY TREATMENTS WITH METHOCEL

Since most watermelon seed will retain only about 0.3 percent of its weight of most dry fungicides, three times this amount was applied by using Methocel sticker. These treatments were compared with ordinary dust treatments (0.25 percent by weight) on both infested and non-infested seed. The bichloride treatments followed by Arasan were also included as an additional control.

Statistical analyses of the survival and disease data showed that there was no significant difference between the dust and slurry treatment means, indicating that the additional fungicide applied was not effective in reducing the incidence of anthracnose (Tables 3 and 4).

Anthracnose control, based on final stand of healthy plants and percentage of seedlings developing anthracnose, was significantly greater where Seedox was used than in all other treatments. This was the only material that came anywhere near eradicating spores of the organism from infested seed. The bichloride of mercury treatment as well as the other dust treatments was unsatisfactory.

A significant interaction occurred between the methods of seed treatment and the fungicide used. Poorer stands resulted in some instances with the slurry treatment, even though more fungicide was applied. For instance, dust treatments with Phygon, Spergon, and Arasan on bichloride of mercury-treated seed resulted in significantly better stands when the same materials were applied at rates of 1 percent by weight in a methocel slurry. On the other hand, while both types of treatments with Seedox were outstanding, the methocel slurry was superior to the dust treatment.

Table 3. The effect of fungicidal dusts and Methocel slurries on final stand of healthy plants[a].

	Mean Stand [b]	
	Dust (.3% by wt. of seed)	Methocel Slurry (1% fungicide by Wt. of seed)
Bichloride of mercury followed by Arasan	57.0	33.3
Phygon	41.9	34.5
Arasan	47.6	45.4
Carbide & Carbon 5400	47.8	50.0
Spergon	57.8	53.5
Dow F-1112	56.3	58.6
Seedox	59.9	66.6
Control	42.1	43.3
LSD between methods of treatment with the same or different fungicides (.05)	5.9	5.9

[a]Seed artificially infested with Colletotrichum lagenarium were used.
[b]Means between methods of treatment were not significant. Interaction between fungicides and method of treatment were significant (.01).

On non-infested seed most of the chemicals performed very well. Captan, Spergon, Arasan, Arasan on bichloride of mercury-treated seed, and Carbide and Carbon 5400 all significantly increased final stand over that of the control (Table 3). No delay in emergence was noted in bichloride of mercury-treated seed.

Table 4. Relation of dust and Methocel slurry treatments to incidence of anthracnose in watermelon seedlings[a].

	Method of treatment b				Mean for Fungicides	
Fungicide	Dust		Methocel slurry			
	Angle	Percentage	Angle	Percentage	Angle	Percentage
Seedox	6.1	1.1	7.5	1.7	6.8	1.4
Dow F-1112	12.5	4.7	18.6	10.2	15.5	7.1
Spergon	17.8	9.3	23.9	16.4	20.9	12.6
Captan	19.3	10.9	24.5	17.2	22.0	14.1
Bichloride of mercury followed by Arasan	21.5	13.4	14.2	6.0	17.8	9.3
Arasan	30.4	25.6	37.7	37.4	34.1	31.4
Carbide & Carbon 5400	38.5	38.8	27.2	20.9	32.8	29.3
Phygon	53.7	64.9	35.9	34.4	44.8	49.7
Control	36.2	34.9	38.2	38.2	37.2	36.5
LSD (.05)					14.8	--
LSD (.01)					20.7	--

For Dust and slurry means of the same fungicide LSD (.05) = 10.0

[a]Artificially infested seed were used.
[b]Dust treatments were applied at 0.3 percent by weight of seed. Methocel slurries contained enough fungicide to equal 1 percent of the weight of tested seed.

PERFORMANCE OF OTHER MERCURIALS

Because higher rates of fungicide failed to give additional disease control, further experiments were performed which included two organic mercurials that had given good anthracnose control in preliminary tests. These data are shown in Table 5. Emmi (10.3% N-ethylmercuri-1,2,3,6-tetrahydro-3,6-endomethano-3,4,5,6,7,7-hexachlorophthalimide), and Panogen (2.1% methyl mercury-dican-diamide) used as soaks at a concentration of 1:1500 for 10 minutes, bichloride of mercury, and Seedox were highly effective in controlling anthracnose, and also resulted in significantly greater emergence when used on non-infested seed. Although in these data these three chemicals appear to have completely eradicated spores of the anthracnose organism, in other tests infected seedlings were observed. Usually, however, they did not exceed 1 or 2 percent. As usual, in all experiments Arasan gave very good results on non-infested seed.

Table 5. The influence of seed treatment fungicides on emergence and incidence of anthracnose on watermelon seedlings.

	Emergence [a]	Percent Seedling infected with Anthracnose [b]	
		Percent	Angle [c]
Seedox	69.6	0	3.3
Emmi	76.8	0	3.3
Panogen	68.6	.5	4.6
Bichloride of mercury followed by Arasan	73.8	0	3.2
Vancide 51	76.6	10.9	19.6
Arasan	68.6	14.9	24.3
Control	66.4	69.2	56.2
LSD (.05)	8.0	-	4.9
LSD (.01)	10.6	-	6.7

[a] Non-infested seed.

[b] Artificially infested seed.

[c] Angle = arc sin $\sqrt{\text{percentage}}$. Where zero percentages occurred they were adjusted to percentage = $\frac{0 + 0.25}{N}$

DISCUSSION

The performance of Semesan, Spergon, and Arasan, which are generally recommended for watermelon seed treatment in the Southeast (5, 6), was disappointing since none of these materials eradicated seed-borne spores in all tests. Furthermore, it was not unusual to observe as great an incidence of anthracnose in seedlings developed from Semesan-treated seed as in control plots (Table 2). Actually, final stand was often below that of the control, suggesting that injury may have predisposed certain seedlings to infection.

In general, bichloride of mercury treatments performed very well in anthracnose control. However, results were inconsistent (Tables 2 and 5), not only in disease control but also under certain conditions emergence was delayed. It is doubtful, however, that under most circumstances emergence would be delayed enough to have any noticeable effect on dates of harvest.

Leach (4) and de Zeeuw and Anderson (1) have found that injury to vegetable seed often occurs with organic mercury compounds particularly when they are used wet. The higher incidence of anthracnose and reduced emergence in plots planted to slurry-treated seeds in these experiments may indicate that the methocel coat made conditions more favorable for phytotoxicity and for germ tube penetration of the seed coat. Rankin (7) has demonstrated that seed coat invasion by C. lagenarium can occur in 24 hours and points out that this may explain why eradication of the organism from the seed is difficult.

Rankin (7) also reports that liquid dip treatments are more effective than dust treatments in controlling anthracnose. He presents no statistical comparisons, however, of the performance of the same fungicide applied at the same rate in both methods. His observations were based initially on the superior disease control obtained with bichloride of mercury soaks when compared with various dust treatments.

In these experiments variation in disease control appears to be related to specificity of the chemical used rather than to rate or manner of application. For instance, Vancide 51 applied as a liquid gave only average results, and bichloride of mercury treatments were sometimes inconsistent, while Panogen and Emmi soaks were highly effective in disease control. Furthermore, Seedox used as a dust treatment was consistently superior in all tests. None of the materials tested in these experiments completely eliminated the organism in all experiments, yet these results were obtained under artificial conditions and spore loads approximating those employed in the tests would not be expected on seed under field conditions. From a practical standpoint such fungicides as Seedox, Panogen, Emmi, and perhaps bichloride of mercury, should prove satisfactory in an anthracnose control program. The data reported herein indicated that they are definitely superior to some of the materials in common use.

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FUNGI ISOLATED FROM LADINO WHITE CLOVER SEEDS (TRIFOLIUM REPENS L.) IN NEW HAMPSHIRE[1]

R. A. Kilpatrick[2]

Abstract

Ladino white clover seeds were harvested from plants heavily infected with different leaf and petiole diseases. Individual seed lots showed 20 to 25 percent injury caused chiefly by the clover seed chalcid. The severity of insect injury varied depending upon maturity of seed in relation to time of puncture. Fungi isolated from insect-injured seeds were essentially the same as those isolated from apparently healthy seeds. Alternaria spp. comprised the highest percentage of total isolates. However, Curvularia trifolii, Pseudoplea trifolii, and Stemphylium trifolii, which cause leaf spots of clovers, were isolated from some seeds.

INTRODUCTION

Seed-borne fungi of Ladino and other white clovers (Trifolium repens L.) have been reported by Hardison (2) and Hopkins (3). Graham (1) cited correspondence from C. M. Leach, who had isolated Stemphylium trifolii Graham from Ladino white clover seed. However, the writer is unaware of any reports on the association of fungi with insect-injured seeds of white clover. The objectives of this study were to determine 1) fungi associated with insect-injured seeds, and 2) kind and relative prevalence of fungi isolated from Ladino white clover seeds harvested from plants heavily infected with different leaf and petiole diseases[3].

MATERIALS AND METHODS

Plants of Ladino white clover attacked by Cercospora zebrina Pass., Curvularia trifolii (Kauff.) Boed., Pseudoplea trifolii (Rostr.) Petr., Stagonospora meliloti (Lasch.) Petr., and Stemphylium trifolii were observed in a nursery at Dover, New Hampshire, in 1956. Several plants were heavily infected by two or three of these fungi. Seeds were harvested from individual infected plants and examined for seedborne fungi.

Seeds in a small Lumite[4] basket were surface-sterilized momentarily in 50% alcohol, then placed in a solution of 2% sodium hypochlorite for 2 minutes. At least 40 seeds from each sample, in lots of five seeds each, were then transferred to eight Petri dishes containing Difco potato-dextrose agar. The seeds were incubated at room temperature (65° to 85° F) for 7 days. Fungi that emerged from the seeds were identified by microscopic examinations.

During this investigation, 4417 seeds, comprising 109 seed lots, were plated on agar. These seeds yielded 1701 fungus isolates. Some seeds yielded more than one fungus.

RESULTS AND CONCLUSIONS

Insect injury (Fig. 1) caused chiefly by the clover seed chalcid (Bruchophagus gibbus Boh.) was common in all seed lots. Individual seed lots showed 20 to 25 percent injury. Light injury usually consisted of a small circular zone surrounding the puncture on nearly mature seeds. Severely injured seeds were shriveled, immature, and often hollow. Numerous punctures were noted on such seeds. Insect damage seemed to be correlated with maturity of

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[2]Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Departments of Botany and Agronomy, New Hampshire Agricultural Experiment Station, cooperating.

[3]Appreciation is expressed to Dr. J. H. Graham for assistance in identifying cultures, and to Dr. J. G. Conklin for identifying the insects.

[4]A synthetic fiberglass screen, 34 mesh, manufactured by Chicopee Mills, Inc., New York.

seed. Alternaria spp. and several miscellaneous fungi were isolated from seeds punctured by insects.

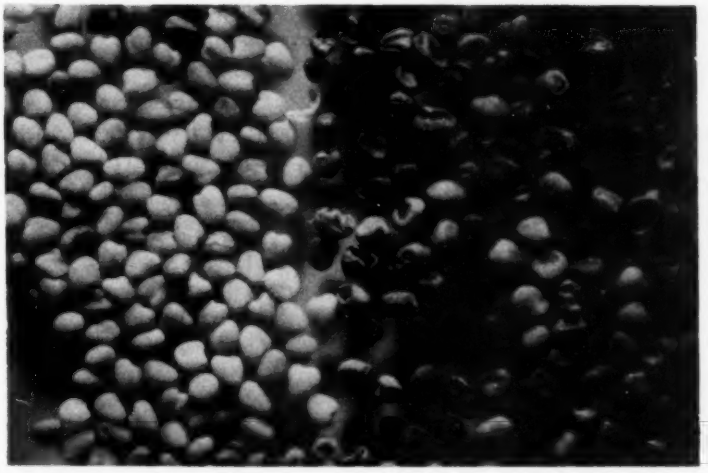

FIGURE 1. Seeds from different clones of Ladino white clover showing, (left) no external symptoms, and (right) insect-punctured and shriveled seeds.

The kind and relative prevalence of fungi isolated from seeds of Trifolium repens are shown in Table 1. Alternaria spp. comprised the highest percentage of fungi isolated from both insect-injured and apparently healthy seeds. Cladosporium spp. and Fusarium oxysporum Schlecht., together, comprised 41 percent of the total isolates. Curvularia trifolii, Pseudoplea trifolii, and Stemphylium trifolii, isolated only in small percentages, are considered of importance on this host. Seeds yielding C. trifolii had been harvested from plants artificially inoculated with this fungus; however, the fungus was never isolated from seeds harvested from naturally infected plants. Both P. trifolii and S. trifolii were isolated from seed obtained from naturally infected plants. Stagonospora meliloti and Cercospora zebrina were never isolated from seeds during this study.

Table 1. Kind and relative prevalence of some fungi isolated from seeds of different clones of Ladino white clover grown at Dover, New Hampshire in 1956.

Fungus	: Percentage of total isolates
Alternaria spp.	53[a]
Cladosporium spp.	26
Fusarium oxysporum	15
Miscellaneous spp.[b]	6

[a]Percentages calculated on the basis of 1701 fungus isolates.

[b]Miscellaneous spp. composed of F. roseum Link, Helminthosporium triseptatum (?), Penicillium sp., Curvularia trifolii, Chaetomium sp., Rhizopus nigricans Ehr., Rhizoctonia solani Kuehn, Phoma spp., Pseudoplea trifolii, Mucor sp., Stemphylium trifolii, and several unidentified species.

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DISEASES OF BIRDSFOOT TREFOIL IN SIX SOUTHEASTERN STATES IN 1956 AND 1957[1]

Charles R. Drake[2]

Summary

The severity of Rhizoctonia foliage blight on birdsfoot trefoil is related to several factors. Most infection developed during warm, rainy weather as the plants approached maturity. In contrast, only minor infection occurred during wet periods when the temperature was below 65°F regardless of age of plants. Also, less infection resulted when plants were cut just before a wet period.

There are apparently unknown factors which seem to influence Rhizoctonia infection. For example, an occasional stand of trefoil survives for 8 or 10 years with only minor infection. However, the progenies of plants in these stands proved to be very susceptible to Rhizoctonia infection when tested at other locations. Hence, some environmental factor or factors appear to play a major role in Rhizoctonia infection.

Root rots of birdsfoot trefoil caused by Fusarium spp., Verticillium spp., and Macrophomina phaseoli attacked plants principally in spaced-plant nurseries. Leafspot and stem canker caused by Stemphylium loti was more damaging in spring and fall when day length was relatively short and the mean temperature low. Other diseases of minor importance were: stem and crown rot caused by Sclerotinia trifoliorum; stem spot and canker caused by Phoma sp.; and Colletotrichum trifolii and C. graminicola which occurred on weakened plants.

INTRODUCTION

In areas where it is adapted, birdsfoot trefoil, Lotus corniculatus L. is a deep-rooted, long-lived perennial. Research at several agricultural experiment stations, however, has shown that it is productive in the southeastern United States usually for only 2 or 3 years, after which the stands become depleted or eliminated by diseases. Longevity of trefoil stands may be increased in the Southeast by learning how to control the various diseases that attack this crop.

During the fall of 1956 and the spring of 1957, surveys were made to determine the cause, prevalence, and severity of birdsfoot trefoil diseases in Virginia, West Virginia, Kentucky, Tennessee, North Carolina and Maryland. The results of these surveys are reported herein.

METHODS

Observations were made on diseases of birdsfoot trefoil at six different agricultural experiment stations and 17 branch stations in the above-mentioned States. Stands from 1 to 11 years old were observed at different seasons to obtain information concerning the important pathogens attacking the crop. In some field plots estimates were made of reduction in stand from diseases.

Disease specimens that could not be identified readily in the field were brought into the laboratory for microscopic observation and isolation.

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[1] Cooperative investigation between the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Virginia Agricultural Experiment Station. Published with the approval of the Director of the Virginia Agricultural Experiment Station.

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RESULTS

Rhizoctonia foliage blight incited by one or more strains of Rhizoctonia solani Kuehn was present on plants in all plots observed. This fungus caused greater and more rapid damage to trefoil than other diseases. It was found on all above-ground parts of birdsfoot trefoil during hot, humid weather from May through September. The fungus usually attacked the lower leaves first, overrunning them with aerial mycelium and causing affected leaves to adhere to stems and branches. If the weather continued hot and humid, entire plants were soon overrun by the rapidly growing aerial mycelium. Stem girdling was also a symptom characteristic of the disease.

At Middleburg, Virginia, Rhizoctonia foliage blight reduced the average stand of the four best varieties (Granger, Italian, Cascade, and Viking) in test plots, from 38 percent when first observed, August 22, 1956, to only an occasional plant by September 28, 1956.

In Eastern Virginia, four varieties (Granger, Italian, Viking and Empire) of birdsfoot trefoil in 96 plots, both in pure stands and in association with three different grasses, Kentucky bluegrass (Poa pratensis L.), orchardgrass (Dactylis glomerata L.), and Kentucky 31 Fescue (Festuca arundinacea Schreb.), were severely attacked by Rhizoctonia foliage blight during the latter part of July, August, September, and October 1956. When the plots were first observed on July 20, 1956, there was an excellent stand, but by November 2, stands were reduced an average of 43 percent.

There was some indication that Rhizoctonia injury to trefoil was reduced at elevations of 3000 feet and above. Only minor foliage blight was detected in a 10-acre field of trefoil at Cransville, West Virginia (elevation 2900 to 3000 feet). Similar observations were made at the Upper Mountain Experiment Station, Laurel Springs, North Carolina (elevation 3000 to 4000 feet).

Root rots were next in importance in causing stand reductions. They were somewhat localized with the more severe infections occurring in spaced-plant nurseries. Three different fungi were associated with the root rots: Fusarium spp., Verticillium spp., and Macrophomina phaseoli (Maubl.) Ashby. The Fusarium spp. and Verticillium spp. were isolated consistently from roots of diseased plants at Laurel Springs, North Carolina; Beltsville, Maryland; Blacksburg, Virginia; and Middleburg, Virginia. The charcoal rot organism, Macrophomina phaseoli, was isolated only from roots of diseased plants at Beltsville, Maryland.

An investigation is now underway to determine which of the above organisms is the primary cause of root rot.

Leaf spot and stem canker incited by Stemphylium loti Graham was present in all plots. Only a few mature plants were damaged by the stem canker phase. However, the pathogen caused severe blighting of young shoots during early March where 4 to 6 inches of stubble overwintered on the plots. Leaves of the apical meristem turned brown and the plants had a stunted or rosetted appearance. New shoots developed from the crown and infected plants recovered rapidly. Stemphylium leaf spot decreased as the day length and mean temperature increased. Similarly, the number of leaf infections increased in the fall as day length and mean temperature decreased. Rapid defoliation followed leaf infection. There seemed to be no appreciable reduction in stand associated with this disease.

Sclerotinia crown and stem rot incited by Sclerotinia trifoliorum Eriks. occurred in isolated areas in plots of trefoil at Blacksburg, Virginia. The only damage from this pathogen was in areas where volunteer red clover was growing in the stand.

Other diseases found on birdsfoot trefoil appeared to be of minor importance. Phoma stem spot or stem canker incited by a Phoma sp. was found on only a few plants in the southeastern tier of States but it is considered potentially important in Missouri.

An investigation is underway to determine if the Phoma sp. that occurs on Lotus corniculatus is the same as that described in 1882 on Lotus scoparius (Nutt.) Ott. as Phoma hosackiae Cke. & Harkn.

Fusarial aerial blight incited by a facultative parasite of the Fusarium group was found only at Laurel Springs, North Carolina. Greenhouse inoculations with this fungus indicated it was not a potential pathogen.

Both Colletotrichum trifolii Bain & Essary and C. graminicola (Ces.) G. W. Wils. occurred on diseased plants of birdsfoot trefoil after the plants had been weakened by other pathogens.

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A RACE OF THE COTTON-WILT FUSARIUM CAUSING WILT OF YELREDO SOYBEAN AND FLUE-CURED TOBACCO[1]

J. K. Armstrong and G. M. Armstrong[2]

INTRODUCTION

During the investigations of races 1 and 2 of the Fusarium causing wilt of cowpeas and soybeans (5), wilted soybeans were collected in 1944 at Hartsville, South Carolina, from which isolates of a Fusarium were obtained that wilted Yelredo soybean but not the cowpea varieties in the experiments. These isolates, 30 and 31, Table 1 (5), were also found to cause wilt of cotton, and of burley and flue-cured tobacco which are not hosts for the races of the cowpea-wilt Fusarium.

Field observations in 1938 (1) indicated that the cotton-wilt Fusarium (F. vasinfectum Atk. or F. oxysporum f. vasinfectum (Atk.) Snyder & Hansen) was probably a cause of wilt of burley tobacco. This was verified by inoculation experiments, which also showed that the cotton fungus caused little or no wilt of a susceptible variety of flue-cured tobacco (3). These results were confirmed by others (8). The host complex for the new race of the cotton-wilt Fusarium is the subject of this report. This race will be designated race 2 and the commonly occurring one, race 1.

MATERIALS AND METHODS

Plants were grown in steamed sand in 2-gallon glazed pots. Roots were cut, and a liquid inoculum was poured around them. All pot experiments were performed in the greenhouse with the temperature control set at 28° C. Details of the procedure are given elsewhere (4)[3]. The fungus isolates were maintained on standard potato-dextrose agar. Mass and monoconidial isolates of both races 1 and 2 were tested.

Inoculations were made on two varieties of tobacco (Nicotiana tabacum L.), burley Kentucky 5 and flue-cured Gold Dollar; a susceptible variety of soybean (Glycine max (L.) Merrill), Yelredo, together with a few pots of the varieties Ottawa, L. Z., Bansei, and Yelnando; 21 resistant or susceptible varieties or breeding lines of cotton, Gossypium hirsutum L.; eight varieties of G. barbadense L.; and the resistant wild cotton, G. thurberi Todaro. The varieties of G. hirsutum included Hurley's Special Rowden, Acala, Empire, Coker 100 Wilt, Deltapine, Stonewilt, White Gold, Super Seven, Hopi, and various breeding lines. Among the varieties of G. barbadense were the susceptible ones Sakel, Sakha 4, Amsak, Pima 32, Bryan, and Tanguis, and the resistant varieties Seabrook Sea Island and Ashmouni. Uninoculated plants were included when a variety was being tested.

Rowden cotton, Yelredo soybean, and Gold Dollar tobacco were also planted for several years in a field plot at Clemson, South Carolina, that was generally infested with race 1 of the cotton-wilt Fusarium, but locally infested in a single area with race 2. Isolations of the wilt Fusaria, which were tested in the greenhouse, were made chiefly from plants in the localized area.

RESULTS

Field Tests

In the first field test in 1946, no wilted plants were observed among approximately 200 Yelredo soybean and 100 Gold Dollar tobacco plants grown in a section of the plot where susceptible varieties of cotton were severely wilted. However, race 2 had been obtained the previous season from a wilted soybean plant in an uncharted location of this field. Consequently, in the second field test, Rowden cotton, Yelredo soybean, and Gold Dollar tobacco were plant-

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[1]Contribution of the Department of Botany and Bacteriology in cooperation with the Cotton and Cordage Fiber Research Branch, Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Technical Contribution No. 276, South Carolina Agricultural Experiment Station.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Plant Pathologist, South Carolina Experiment Station, respectively.

[3]Misprint in Phytopathology 38: 809. 1948. KH_2PO_4 should be 0.008M and $Ca(NO_3)_2$ 0.0356M.

Table 1. Results of inoculations of cotton, soybean, burley and flue-cured tobacco with races 1 and 2 of the cotton wilt Fusarium[a].

Sources of isolates	Soybean Yelredo				Flue-cured tobacco Gold Dollar				Cotton Rowden				Burley tobacco Ky. Burley 5			
	Number of isolates	Number of tests	Number of plants	Percent external wilt	Number of isolates	Number of tests	Number of plants	Percent external wilt	Number of isolates	Number of tests	Number of plants	Percent external wilt	Number of isolates	Number of tests	Number of plants	Percent external wilt
RACE 2																
Soybean																
Hartsville	9	26	327	67.6	5	13	66	42.4	9	21	241	97.9	3	7	35	94.3
Clemson	3	3	46	41.3	22[b]	43	295	58.3	3	6	79	93.6	1	2	10	100.0
Clemson					10	10	56	67.9								
Tobacco																
Flue-cured																
Clemson					4	4	23	52.2	5	6	65	100.0				
Cotton																
Clemson	3	4	67	40.3	6	6	24	70.8	3	3	35	97.1	2	2	10	100.0
RACE 1																
Soybean																
Clemson	3	2	28	0					3	3	28	92.9				
Cotton																
Clemson	5	5	73	2.6	19	19	90	5.6	19	19	247	93.7				
Ala., Ark., Ga., N.C., Okla., S.C., Tenn.	17	17	216	5.5	5	5	33	9.1	22	24	336	93.2				

[a]Uninoculated plants which remained free of disease were included in each experiment.
[b]Nineteen of these isolates came from single spores from the three original ones pathogenic on cotton.

ed in alternating rows throughout the field. The cotton plants wilted throughout the plot, but wilted and dead tobacco and soybean plants were found only in a restricted section about 50 feet long and a few rows wide. Additional tests during the intervening years have shown that race 2 is still confined chiefly to this limited area, which is also infested with race 1.

When soybean or flue-cured tobacco have been planted subsequently in other sections of the field, an occasional plant has shown symptoms of wilt, but the disease was not severe. That the slight symptoms of wilt were due to race 1 was proven by greenhouse inoculations with isolates from soybean (Table 1). No attempts were made to isolate race 1 from tobacco in this series of experiments.

Greenhouse Tests

1. Yelredo Soybean, Gold Dollar and Kentucky 5 Tobacco, and Rowden Cotton: The results of the inoculations with isolates of races 1 and 2 on these hosts are given in Table 1. Only external symptoms of disease are considered in the Table. Many plants that did not show external symptoms exhibited mild to severe internal discoloration, and the wilt Fusarium could be recovered from them. In several instances, mass isolates were used along with the single-spore cultures derived from them, but no significant differences in pathogenicity were noted.

Race 2 was obtained from soybean, Gold Dollar tobacco, and cotton at Clemson, but only from soybean at Hartsville. The isolates from plants at both locations caused wilt of the four hosts listed in Table 1, but they were generally more pathogenic on cotton and burley tobacco than on soybean and Gold Dollar tobacco. Recently isolated cultures usually produced fairly high percentages of wilt, but they varied in pathogenicity on the last two hosts. In the first tests on soybean, each of four single-spore isolates caused 100 percent wilt, whereas two others gave 50 and 41.7 percent wilt, respectively. Similar variations were evident on Gold Dollar tobacco. These high percentages of wilting are not evident in the Table, since all the results for one isolate-host combination are given in the averages.

The pathogenicity of some of the most virulent isolates was greatly reduced or lost on soybean and Gold Dollar tobacco after being maintained in culture, although this was not true on cotton. For example, a monoconidial isolate which was tested on Gold Dollar tobacco immediately and a year later produced 86.7 and 27.3 percent wilt, respectively. Another monoconidial isolate on the same host in four pot tests the first year, seven the second year, and three the seventh year produced 100, 67.6, and 28.5 percent wilt, respectively.

Twenty-two monoconidial cotton-wilt isolates, from seven southern States, which were highly pathogenic on Rowden cotton, were tested on either Yelredo soybean or Gold Dollar tobacco to see whether the new race might be widely distributed (Table 1). Thirteen of the isolates were from various sections of South Carolina; two each from Georgia, North Carolina and Oklahoma; and one each from Arkansas, Alabama, and Tennessee. Only a few soybean and tobacco plants showed slight external symptoms of wilt in the greenhouse experiments. Since occasional soybean or tobacco plants growing in the field infested with race 1 show a similar slight reaction, it was evident that race 2 was not found among these isolates.

2. Other Soybean Varieties: A total of 64 plants of the varieties, Bansei, Ottawa, L. Z., and Yelnando in seven pots were inoculated with virulent cultures of race 2 from soybean. Only three plants in all the pots showed slight symptoms of wilt, an indication of high wilt resistance, which is similar to that for race 1 of the cowpea fungus (5).

3. Other Cotton Varieties: Twenty-nine varieties or breeding lines of cotton, some of which are listed above, had been tested in other experiments for their relative susceptibility or resistance to race 1 of the cotton-wilt fungus. Since it was desirable to know their reaction to the new race, inoculations were made with isolates of race 2 from either or both soybean and tobacco. In these tests, there were usually two pots of ten plants each of a variety or line, although in some cases, there were as many as ten pots. In general, their susceptibility or resistance to race 2 was similar to that to race 1.

4. Other Genera: Isolates of race 2 have not been tested on the numerous genera and varieties that are usually used in the cross inoculations performed in this laboratory. However, the inoculation of plants such as mimosa, Criollo sesame, Bonny Best tomato, Lady Finger and California Blackeye 5 cowpeas, and Heavenly Blue aster, indicates that, except for soybean and flue-cured tobacco, the host range is the same as for race 1. Race 1 of the

cotton-wilt Fusarium causes severe wilting of susceptible varieties of American Upland cotton, okra, burley tobacco, and alfalfa, and mild wilting of the weed Cassia tora L.

DISCUSSION AND CONCLUSIONS

The new race has been found only at locations 200 miles apart, viz., Hartsville, South Carolina, in the Coastal Plain, and Clemson, South Carolina, in the Piedmont. A study of the host relationships has led to the conclusion that it is a cotton-wilt Fusarium with a wider host range than race 1, and that at Clemson it is restricted to a small area in the larger wilt field. Since some isolates of race 1 of the cotton fungus cause wilting of a few plants of both Gold Dollar flue-cured tobacco and Yelredo soybean, perhaps race 2 is a mutant with increased pathogenicity on these two hosts. A wider distribution of this race may be revealed if susceptible varieties of either soybean or flue-cured tobacco are grown in other fields infested with the cotton-wilt fungus.

The factor or factors that determine pathogenicity on soybean and tobacco seem unstable, since pathogenicity on both hosts may be greatly reduced or lost in relatively short periods, although this has not occurred on cotton.

That many of the wilt-Fusaria of the section Elegans are not specific in pathogenicity on a single or closely related host has been reported by the authors in several abstracts and papers. The results presented here show further complications for the rigid "formae" system of classification (9). The cowpea (5) and cotton Fusaria are shown to be related, since the Yelredo soybean is a common host for race 1 of the cowpea Fusarium and race 2 of the cotton Fusarium. It should be recalled, however, that the two races of the cotton Fusarium do not cause wilt of cowpea, nor do the races of the cowpea fungus cause wilt of cotton. Furthermore, the sweet potato Fusarium (7) and races 1 and 2 of the cotton fungus are specific in pathogenicity on the host from which they were obtained, but all of them will cause wilt of a susceptible burley tobacco. A relationship between the alfalfa wilt fungus (6) and both cotton races is indicated by alfalfa as a common host.

The investigation of the wilt Fusaria began with a large collection of isolates of the cotton wilt organism. When monoconidial lines were grown on several media, the variability of these in morphological and cultural characters was so pronounced that their identification according to Wollenweber and Reinking (10) was impossible. Monospore subcultures through 17 generations also revealed great variability (2). By 1940, it was known that the idea of rather strict host specificity (9) was invalid; therefore, a comprehensive investigation of a large number of the wilt Fusaria to determine the host relations was necessary as a basis for a system of classification. Since that time, 37 different wilt Fusaria have been tested in numerous cross inoculations, using single-spore isolates and growing the plants in sterilized sand. A large amount of data has been assembled for a revision of the section Elegans.

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COTTON AND CORDAGE FIBER RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH THE DEPARTMENT OF BOTANY AND BACTERIOLOGY, SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION

THE EFFECT OF SOIL TYPE AND SOIL TEMPERATURE ON THE GROWTH AND DEVELOPMENT OF WITCHWEED (STRIGA ASIATICA) UNDER CONTROLLED SOIL TEMPERATURES[1]

R. R. Nelson[2]

Abstract

Data are reported on the effect of soil type and soil temperature on the establishment and development of witchweed, Striga asiatica, on corn under controlled soil temperatures in the greenhouse. At a constant soil temperature of 32° C; the parasite developed to maturity in pots of sand, sandy loam, loam, sandy clay loam, clay loam, light clay, and peat soils, but not in heavy clay. Witchweed plants appeared aboveground in light sand, sandy loam, and loam soils at a soil temperature of 25°; in sand and sandy loam soils at 22°; but only in light sand at 18°. Witchweed flowered in all soil types studied at soil temperatures of 32° and 36°. At 28°, it flowered in light sand, sandy loam, and loam soils, but at 25°, flowered only in light sand, and did not flower in any soil type at 18° or 22°. The number of days needed for emergence and subsequent flowering was considerably greater in the heavier soils than in light soils at soil temperatures of 28°, 32°, and 36°. Plants did not flower in the heavier soils below 28°. The studies indicate that soil type and soil temperature will be among the important limiting factors in the potential spread of witchweed.

INTRODUCTION

Witchweed, Striga asiatica (L.) Kuntze, is a parasitic flowering plant that attacks the roots of a wide variety of cultivated and wild gramineous hosts in several tropical and subtropical areas of the Eastern Hemisphere. In the Western Hemisphere witchweed was first discovered on corn (maize) and a number of grassy weeds in North Carolina in 1956, and subsequently in South Carolina (2). Since that time, research has been undertaken to determine the behavior and growth habits of the parasite in the United States, with special emphasis on ultimate control or confinement of the parasite.

Soil type and soil temperature appear to be important factors affecting the geographic distribution of S. asiatica (1, 3, 4, 6, 7). Witchweed apparently thrives best in light sandy soils, but it also may persist in heavier soils with increased difficulty. It was considered advisable to conduct controlled experiments on the effect of soil type on the development of S. asiatica and to correlate the combined effects of soil type and soil temperature.

MATERIALS AND METHODS

Fourteen soil samples, representing eight types, were used in these studies. Eight of the soils constituted surface samples of the following important soil types present in North Carolina: Lakeland fine sand, Appling sandy loam, Norfolk sandy loam, Appling loam, Appling sandy clay loam, Appling clay loam, Appling light clay, and Cecil heavy clay. Six additional soils were obtained from the Midwest Corn Belt. These samples represented Clarion loam, Barnes loam, Skyberg sandy loam, Port Byron sandy loam, Nicollet clay loam, and peat.

The development of Striga asiatica in the eight soil types was studied in controlled temperature tanks at a soil temperature of 32° C, optimum or near optimum for germination of witchweed seed (5). The tanks were used in a controlled temperature room at a constant air temperature of 65° F.

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the North Carolina Agricultural Experiment Station.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

Plants were grown under an artificial light intensity of 400 to 500 foot-candles. Corn (Zea mays L.) and Johnson grass (Sorghum halepense (L.) Pers.) were used as hosts in each of the 14 soil samples. Seed of witchweed harvested in the fall of 1956 was added to the soils at the time of planting. The eight soil types were tested concurrently in a greenhouse at a soil temperature of approximately 32° C. The experiments both in the temperature tanks and in the greenhouse were repeated.

An attempt to evaluate the comparative effects of soil types was based on the number of days needed for S. asiatica to appear aboveground and on its subsequent growth and development.

Soil types were tested at 18°, 22°, 25°, 28°, 32°, and 36°C, in controlled temperature tanks at an air temperature of 65° F. Temperatures within a deviation of ± 1° C were recorded daily.

EXPERIMENTAL RESULTS

Effect of Soil Type on the Development of Witchweed

At 32° C, Striga asiatica grew readily and developed to maturity in seven of the eight soil types. Plants did not appear aboveground in heavy clay in any of the tests. When the heavy clay was carefully washed away from the host roots, no immature plants were observed, indicating that parasitic contact had not occurred. It is not known whether the seed germinated in heavy clay.

The initial emergence of the parasite aboveground is favored by lighter soils, although the differences among soil types are not so marked as published reports might suggest (Table 1). Light sand, sandy loam, and loam soils supported initial development of witchweed equally well. The time required for witchweed to emerge in clay loams was considerably greater. Although the plants flowered and set seed in all soil types but heavy clay, the rate of development and vigor of the parasite was significantly poorer in the heavier soils. The plants in clay loam soils were considerably smaller, and frequently flowered and set seed at a height of 2 to 3 inches.

Effect of Soil Temperatures on the Development of Witchweed

Soil temperatures had a marked effect on the initial establishment of witchweed in all of the soil types studied (Table 2). The plant appeared aboveground in all soil types tested at temperatures of 32° and 26° C. At 28° C, it was established in all of the soils tested except Appling light clay. At temperatures below 28°, the parasite emerged only in the lighter soils, including sand, sandy loam, loam, and sandy clay loam, at 25° C. Only light sand supported initial growth at 18° and 22° C.

The effect of soil temperatures on the subsequent development of witchweed was even more pronounced than on successful establishment of the parasite. Plants of witchweed flowered in all soil types at 32° and 36° C. At 28° witchweed flowered only on light sand, sandy loam, and loam, and at 25° C produced flowers only on light sand. The parasite did not flower in any soil type at 18° and 22° C.

Soil temperatures had a marked effect on the length of time required for witchweed to emerge in the different soil types. At 32° and 36° C light sand, sandy loam, and loam soils supported the initial establishment of plants equally well, while initial appearance in the heavier soils was delayed considerably (Table 2). The number of days needed for plants to appear increased progressively with decreases in temperatures below 32° C in all soil types.

The length of time required from emergence to flowering also increased proportionally with the time required for emergence in the various soil types at different temperatures. Plants grown in sand, sandy loam, and loam soils flowered in approximately 3 weeks after emergence at 32° and 36° C, while at 28° 4 weeks were required in sandy soil and 5 weeks in sandy loam and loam.

Plants grown in the heavier soils flowered in 4 to 5 weeks at 32° and 36° C, and in approximately 6 weeks at 28°. At 25° C, plants flowered only in light sandy soil, and this required approximately 6 to 7 weeks. Witchweed did not flower in any soil type when grown at 18° and 22° C.

Table 1. Number of days needed for witchweed to emerge in different soil types at a soil temperature of 32° C.

Soil type	Number of days for first plant to emerge
North Carolina Soils	
Lakeland light sand	28
Norfolk sandy loam	31
Appling sandy loam	33
Appling loam	34
Appling sandy clay loam	41
Appling clay loam	44
Appling light clay	53
Cecil heavy clay	--
Midwest soils	
Skyberg sandy loam	34
Port Byron sandy loam	30
Clarion loam	34
Barnes loam	37
Nicollet clay loam	40
Peat	47

Table 2. The effect of soil type and soil temperature on the number of days needed for the first witchweed plant to appear aboveground. [a]

Soil Type[b]	Soil temperature (° C)					
	18	22	25	28	32	36
Light sand	70	60	50	40	30	30
Sandy loam	-[c]	75	60	50	30	30
Loam	-	-	65	50	35	35
Sandy clay loam	-	-	65	55	40	40
Clay loam	-	-	-	60	45	45
Peat	-	-	-	60	50	50
Light Clay	-	-	-	-	55	55

[a] Because the length of time needed for the plants to appear varied among replications and between tests, the number of days listed in the table are, at least in part, relative. The length of time (30 days) for plants to appear in light sand at 32° C is considered the base to which other figures are compared. Although in many instances the figures represent approximate averages, the number of days stated are intended to be comparative.

[b] The number of days required for plants to emerge in North Carolina and Midwest samples of the same soil type was similar. The number of days listed are considered representative of all soil samples.

[c] Designates that witchweed did not become established.

DISCUSSION

The present studies indicate that soil type and soil temperature are important factors influencing the growth of witchweed and may limit the potential distribution of the parasite in the United States. These preliminary studies were under constant controlled conditions and dealt with only two growth factors. They do not provide a basis for predicting the ultimate potential spread of the parasite. The reaction of witchweed to soil type and soil temperature indicates that initial establishment might occur in some of the major corn-growing areas, but these studies were not designed to determine whether or not the parasite would develop to maturity under Corn-belt conditions. However, if it should be introduced into certain areas of the Southwestern United States, where high soil temperatures prevail throughout a large part of the growing season, the results of these studies suggest that witchweed might become a serious problem.

Although soil types and soil temperatures appear to be of prime importance for witchweed development, other factors undoubtedly need to be considered. Soil moisture, organic matter content, soil fertility, day length, and light intensity might affect the establishment and subsequent development of the parasite. Soil temperatures undergo a seasonal trend with some fluctuation. Temporary soil temperature drops below the optimum average probably would retard the development of the parasite. Soil temperatures slightly above the optimum appear to have no detrimental effect on plant development.

The existence of physiological strains of Striga hermonthica (Del.) Benth. has been suggested (3). In the Sudan of Africa, sorghum is severely attacked by S. hermonthica in clay soils, while Pennisetum millets are practically immune. The reverse is true in light sandy soils in other areas of the Sudan. It is conceivable that similar genetic variation also exists in S. asiatica.

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DEPARTMENT OF PLANT PATHOLOGY, NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

UNUSUAL RECORDS OF PLANT DISEASE OCCURRENCE

MASTIGOSPORIUM ALBUM RIESS ON ALOPECURUS PRATENSIS: A CORRECTION

By I. L. Conners[1]

When Johnson[5] recently recorded in the REPORTER the occurrence of Mastigosporium album Riess on Alopecurus pratensis L. at Peggy's Cove, Nova Scotia, he quite correctly attributed the first report of this fungus in North America to Ross[6], who collected it near Ithaca, New York, in June 1954. However, Johnson quite overlooked the later reports of Creelman[2,3,4], who actually collected the fungus in a field of meadow foxtail at Wolfville, Nova Scotia, on June 15, 1953, a season before Ross, although his report to the Canadian Plant Disease Survey[2] was not published until December 1954. A portion of the original collection is deposited at Ottawa (DAOM 38810). Collections are also deposited in the Plant Pathology Laboratory, Kentville. Johnson's collection at Peggy's Cove is of interest because Creelman[2] mentions that he looked for the fungus elsewhere in Nova Scotia in 1954 without success.

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[2]Canadian Plant Disease Survey, Ann. Rept. 33(1953). 1954, p. 50.

[3]Ibid, Ann. Rept. 34(1954). 1955, p. 52.

[4]Creelman, D. W. 1956. The unusual occurrence of three leaf-spotting fungi on grasses in Nova Scotia. Plant Dis. Reptr. 40: 510-512.

[5]Johnson, A. G. 1957. Mastigosporium album Riess on Alopecurus pratensis in Nova Scotia. Plant Dis. Reptr. 41: 949.

[6]Ross, J. P. 1954. Mastigosporium album Riess on Alopecurus pratensis in New York. Plant Dis. Reptr. 38: 607-608.

TWO UNUSUAL APPLE FRUIT ROTS IN PENNSYLVANIA

By Harry C. Fink[1]

Two unusual fruit rots were found in one storage house in Pennsylvania in 1956-57.

Stayman apples were severely attacked by Phytophthora cactorum (Leb. & Cohn) Schroet. The fruit had been placed on straw under the trees to promote increase in color after harvest. The fungus apparently grew from the soil through the damp straw and infected the fruit. The rot was not apparent until after the fruit had been in storage for some time. The grower reported a $10,000 loss due to disfigurement of otherwise marketable fruit and increased cost of repacking before sale.

Cephalosporium carpogenum Ruehle was isolated from Golden Delicious apples. The symptoms of the rot and measurements of the isolated fungus fit the description by Ruehle[2] for the disease and pathogen. These fruits were taken from storage, where they had been placed immediately after harvest. This is the first report of this disease in Pennsylvania.

PENNSYLVANIA STATE UNIVERSITY

[1]Associate Professor Plant Pathology, Botany and Plant Pathology Department, The Pennsylvania State University.

[2]Ruehle, George C. Phytopathology 21: 1141-1152.

CORRECTIONS

REPORTER, December issue (Volume 41, Number 12), page 1029: The correct chemical name for "Cyprex", according to the American Cyanamid Company, the manufacturers, is n-dodecylguanidine acetate. The name is misspelled in the first lines of both summary and introduction. Also, in Table 2, page 1031, line 14 should read "ferric sulfate 1/2 pound" instead of "ferbam 1/2 pound."

SUPPLEMENT 248, page 111, Footnote 3: The footnote should read "The hosts include many winter-grown vegetables raised commercially in Florida." The footnote as given refers to accumulated knowledge, not to results of the authors' tests.

ATTENTION, PLEASE!

WANTED: INFORMATION CONCERNING CERTAIN CHARTER MEMBERS OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

Dr. Glenn S. Pound, Chairman of the Fiftieth Anniversary Committee of the American Phytopathological Society, wants to have an accurate listing of living Charter Members at the time of the Golden Jubilee celebration. He has not been able to locate sixteen, listed below. If any reader possesses any information on the whereabouts of any of these persons please send it to Dr. Pound at the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

The names are as follows:

Dorrance, Anne
Field, Ethel C.
Garman, Harrison
Gilbert, A. H.
Harding, H. A.
Hart, W. O.
Humphrey, C. J.
Lewis, C. E.
Markham, Frances G.
Olive, E. W.
Rand, F. V.
Spaulding, Perley
Speare, Alden
Snyder, Clifford D.
Wallace, Errett
Wood, Anna K.

BOOK REVIEW

"PRINCIPLES OF PLANT PATHOLOGY", by E. C. Stakman and J. George Harrar. xi + 581 pages. 1957. The Ronald Press Company, 15 East 26th Street, New York 10, New York. Price: $8.00.

This book will be welcomed because, essentially, it epitomizes the senior author's long career as investigator and teacher, in which he has attained international renown. Fundamentally, it appears that the book is largely an expression of Dr. Stakman's concepts and judgments, thought out, tested, and revised through many years of research and teaching.

Over and over again the authors stress how little we really know and how much we need to know, and point out where we should look for knowledge and the type of research needed to get it. Scarcely a section has not at least one statement, usually several, similar to these: "the present state of relative ignorance", "much is still left to conjecture", "too little is known; much remains to be learned", "still require explanations that can be furnished only by basic researches", "acute pathological problems continue to develop", "what happens is definitely known, but why it happens" is not, and innumerable others. Of course showing what needs to be done as well as what has already been accomplished should be one of the book's major functions, and it is admirably fulfilled. In addition to this constant statement of specific questions, a chapter is devoted to future research.

As one would expect from both authors, the international aspects of plant disease importance, control, and research receive special attention, not only in two separate chapters but, again, throughout the book. To the authors, "plant health is basically an international problem."

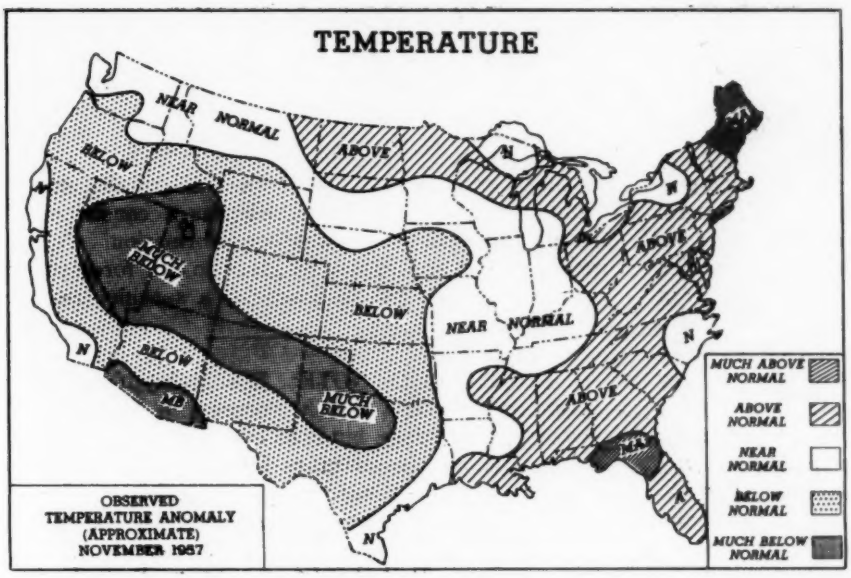
The style is excellent, being easy to read, clear, and interesting, scientific but not unnecessarily technical and not condescending. Definitions are given as terms are used. There is a good statement of the function of definitions in science: "Definitions of scientific words or phenomena are not so much ends in themselves as attempts to group similar phenomena and describe them as clearly as possible so that they may mean essentially the same thing to different people." (page 74).

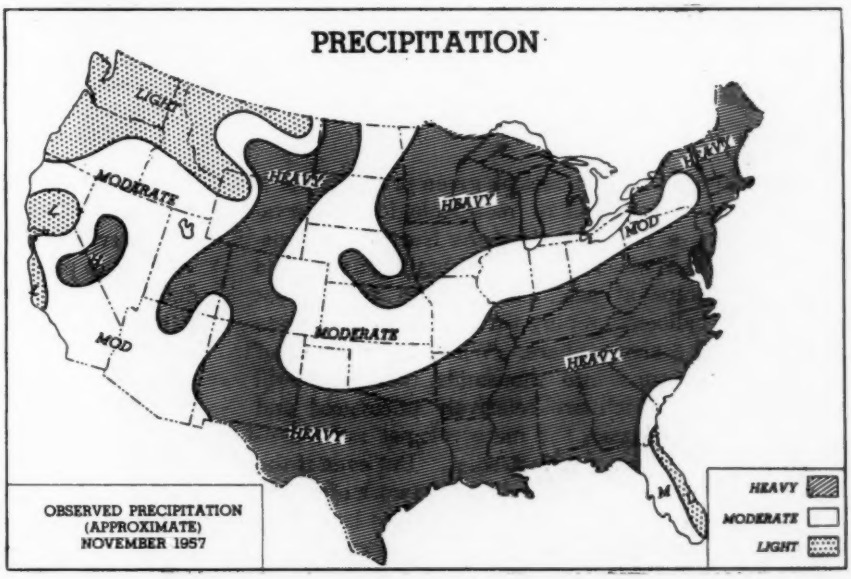
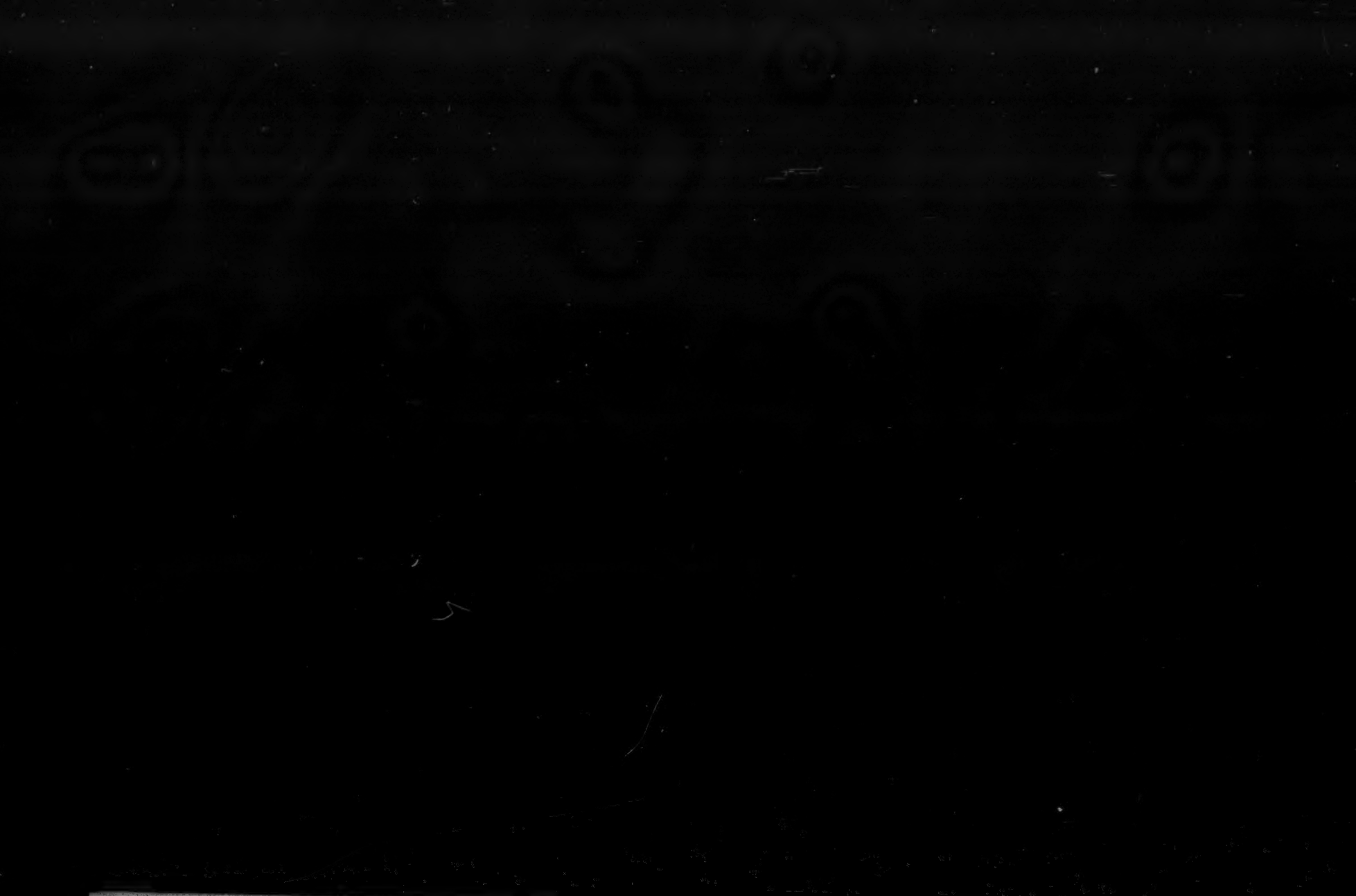
Frequently a whole subject is illumined by a vivid expression like the one that really starts the book, "Man occupies this planet as a guest of the plant kingdom." It would be hard to explain more forcefully the fundamental reason for the importance of plant pathology.

Plant pathologists will be glad to have this organized discussion of the present state and future needs of their science. Because of its wide scope and forward look it will be valid for a long time to come.

The principles of the title are outlined in the Chapter headings. After a short introduction, Chaper 1 starts with the Importance of Plants and Chapter 2 follows with the Importance of Plant Diseases. Chapter 3 discusses the Nature and Classification of Plant Diseases, Chapter 4 the Causes of Plant Diseases, and Chapter 5 the Nature and Classification of Plant Pathogens. Then Chapter 6 takes up the Growth and Reproduction of Plant Pathogens, Chapter 7 the Genetics of Plant Pathogens, Chapter 8 the Production and Liberation of Inoculum, Chapter 9 the Dissemination of Plant Pathogens, Chapter 10 the Phenomena of Infection, and Chapter 11 the Effect of Environment and Nutrition on Disease Development. Various aspects of plant pathology are the subjects of the remaining chapters: Chapter 12 concerns Plant Diseases of International Importance; Chapter 13, Diseases in Transit and Storage; Chapter 14, Quarantines, Eradication Campaigns, and International Plant Protection; Chapter 15, Cultural Practices in Disease Control; Chapter 16, Chemical Control; Chapter 17, Resistant Varieties. Finally Chapter 18 forecasts Future Problems and Prospects. Brief bibliographies follow each chapter and Appendix A at the end of the book lists Important Books in Plant Pathology. The Latin names of the principal pathogens and principal insects cited in the text are also listed in appendices. Besides the Subject Index there is an alphabetical index of investigators whose work is cited.

The illustrations are almost all new, original, and excellent. -- JESSIE I. WOOD





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.